

6 March 1980

No easy route to non-proliferation

NOWHERE has the interrelationship between politics and technology become more entangled than in discussing the relationship between the spread of nuclear energy and the proliferation of nuclear weapons. To candidates in the 1976 US presidential campaign, the issue seemed relatively straightforward: the greater the availability of plutonium — either through the reprocessing of spent nuclear fuel or through the deployment of fast breeder reactors — the greater the chance that some of this would be diverted to military purposes. Domestically, this philosophy became relatively easy to institutionalise in the Nonproliferation Act of 1977. Internationally it has fallen on sceptical ears.

The final report of the International Nuclear Fuel Cycle Evaluation (INFCE), initiated by President Carter over two years ago in an attempt to generate an international consensus behind the US strategy, has ended up a long way from endorsing his position. Admittedly there are parts of the final report from which the US can take satisfaction. For example, the nations taking part in the study agreed in general that sensitive nuclear facilities capable of producing weapons-grade material, such as reprocessing and enrichment plants, should be limited to as few countries as possible. And the report also confirms the US view that reprocessing is not a prerequisite for the disposal of nuclear wastes, and of marginal economic significance for thermal reactors.

US negotiators are also claiming solace from the fact that, although they have been unable to secure agreement on slowing down the deployment of fast breeders in the interests of non-proliferation, they have at least placed the issue more prominently than before on the international agenda. But this victory is, in many ways, a Pyrrhic one. INFCE did not agree to support any generalisations about the comparative proliferation risks of different fuel cycles. Indeed during its deliberations, the international deployment of fast breeder programmes, particularly in countries such as France concerned about guaranteed future access to uranium supplies, seems to have increased.

What the debate has lacked is a climate of trust. And this is as true for the relationship between the US and its fellow nuclear suppliers as between the suppliers and their customers. Many countries were upset, not so much by the US strategy, but by the apparent strong arm tactics by which it was seeking to impose it unilaterally. President Carter declared his intention that the US, "Should provide strong leadership, using our own exports of nuclear fuel and technology to persuade other countries not to seek or sell the technology to make bombs." Many took exception to this attempt to use economic hegemony to force the rules of the game covering international trade in nuclear technology — particularly at a time when the US and European industries

seemed about to compete for lucrative third world markets.

The value of INFCE is that it has placed many of the issues raised by President Carter in broader perspective. We now know, for example, that there is unlikely to be any one technical fix to the proliferation question, and that the thorium-cycle does not necessarily offer any great advantages in this respect. We also know that the lack or adequacy of uranium supplies cannot be demonstrated simply. But whether we are any closer to reducing the dangers of proliferation is unclear.

One problem is that, whatever the detailed conclusions and recommendations of the report, any message that fast breeders are safe could, unless carefully handled, lead to unwarranted complacency. The report has successfully argued that political issues with regard to restricting proliferation cannot be reduced to technical questions relating solely to the availability of fuels or reactor technology. But it conveys an optimism about the ability of political institutions to cope with the problems that may, in practice, turn out to be equally overstated.

Perhaps this optimism is no greater than that with which President Carter swept into office three and half years ago, determined to demonstrate that controlling the hazards of nuclear power — or for that matter eliminating infringements of human rights — was little more than a question of political will. But such arguments were conceived in the calmer days of the mid-1970s, when the short-term stability of international relations made it possible to argue in terms of long-term strategies.

In the aftermath of Iran and Afghanistan, the climate has changed dramatically. Yet the irony is that, just at a time when the US is trying to restrain countries such as Pakistan and India from a rush to nuclear weapons, and arguing the need for effective international controls, it is engaged with other western nations in boosting its own nuclear arsenal.

Given this situation, it is difficult to see emerging the "Bargain of confidence" urged last month by a study group convened by the Royal Institute for International Affairs, with support from the Rockefeller Foundation. Indeed it is the reality of self-interest that needs to be addressed if we are to make genuine progress towards reducing the threat of nuclear war. Just as there are no technical fixes to the proliferation problem, so we should not be fooled into thinking there are any quick political fixes.

Doing what we can to raise the global standard of living, and reducing the tensions that flow from inequities in the distribution of wealth and power, will in the long run be the only way of removing the incentive for nuclear war. In the short term, efforts to conclude a comprehensive ban on the testing of new weapons, and to maintain pressure for arms limitation treaties such as SALT 2, would be a welcome show of good faith. A world built on conflicts and tensions — with or without fast breeders — can only encourage the instabilities that point to nuclear disaster. □



United Kingdom

Chemical company suppresses dioxin report

WORKERS exposed to 2,3,7,8-tetrachlorodibenzodioxin (dioxin), between 1968 and 1971, during manufacture of the herbicide 2,4,5-T, at the Derbyshire site of the UK company Coalite and Chemical Products Ltd, could face an increased risk of developing cardiovascular complaints, according to confidential documents in *Nature's* possession.

The documents — reports of detailed clinical and laboratory investigations on a number of dioxin-exposed workers at Coalite — suggest that the company has been falsely reassuring about the health of this particular sector of its workforce. According to a spokesperson for the Health and Safety Executive (HSE), the Executive also believes that the Coalite workforce has no long-term health problems. However, its view is based only on reassurances given by the company and not on a study of the medical reports which Coalite has not published and which the HSE is powerless, in law, to demand from the company because it no longer manufactures the product, 2,4,5-T.

Ironically, it was pressure from the HSE which forced Coalite to carry out the investigations in the first place shortly after the Séveso accident in 1976. Included in the study were 126 individuals, 41 of whom were known to have been exposed to dioxin, and showed symptoms of chloracne; 54 of whom might have been exposed to the chemical; and a supposed control group of 31. One of the studies compared the blood chemistry of the different groups. The author of the report of that study is Dr Jenny Martin, a lecturer in Occupational Medicine at the University of Manchester and a consultant chemical pathologist at Chesterfield Royal Hospital when the study was commissioned.

The results of that study show that the dioxin-exposed group has a greater incidence of impaired liver function as measured by the enzyme gamma-glutamyl transpeptidase. Furthermore when the results for serum cholesterol, triglyceride, high density lipoprotein etc were subjected to multivariate analysis — under the guidance of the Department of Probability and Statistics at the University of Sheffield — they showed a significant difference between the dioxin-exposed group and the controls. In the dioxin-exposed group, levels of serum cholesterol and triglyceride were higher and high density lipoprotein lower than in the controls. These are factors commonly held to imply an increased risk of cardiovascular disease.

None of this information is to be published, however. According to Martin, Coalite decided not to publish it on the

advice of Dr Kenneth Crow, a consultant dermatologist at St Margaret's Hospital, Swindon. She claims that Crow had told her that he had advised Coalite not to publish on the grounds that he was not happy with the statistics used in the report.

Crow, one of the world's leading authorities on chloracne, was involved in the treatment of such cases at Coalite. When approached by *Nature* to comment on the study, he said that he did not know which study was being referred to and that he would have to see it before he could comment. However, he strongly denies ever having advised Coalite not to publish the results of any study. *Nature* has approached Coalite for its side of the story, but with no success. Coalite stopped talking to journalists shortly after the Séveso accident in 1976.

Some related information, however, has been published. Instead of using an age and occupation-matched control group, Coalite chose to bolster the number of controls by including management staff undergoing a regular lipid screen at the time. When the study was commissioned, Martin was unaware of the composition of the three study groups. When she later learnt that the control groups had not been properly matched, she arranged to re-examine eight of the Coalite workers suffering from chloracne and to compare their blood chemistry with a matched control group.

The results of this second investigation, published in a letter to the *Lancet* (24 February, 1979, page 446), also show increased serum cholesterol and reduced serum high density lipoprotein in the dioxin-exposed group. The differences were considerably more marked than in the original, larger study. However, they were not statistically significant, a point which Martin notes, but says is simply due to the small numbers of subjects involved.

Perhaps the most extraordinary aspect of the story, however, concerns a burglary at Martin's house. Shortly after publishing her letter in the *Lancet*, Martin's house was broken into and the detailed medical records of the eight Coalite subjects were removed from her filing cabinet. Martin reported the theft to the local police constabulary but as she had no idea why anyone would want to steal this information the police investigation never got off the ground. A police spokesperson at Macclesfield confirms that the theft had been reported, that the case was not closed, but that as there was no clues about the motives for the theft, it was unlikely that it would ever be solved.

Martin told *Nature* that she had been

very distressed when she discovered the theft of the material for the second survey. She has no duplicate copy so the work is now lost.

She was surprised that *Nature* had obtained a copy of her original Coalite report, and having confirmed its authenticity said she was most unhappy that Coalite were not publishing the data. She said the company's decision was a major reason for her carrying out a second study and reporting it to the *Lancet*.

Coalite did, however, release an abbreviated form of the original report to one of the unions involved with the workforce at its Bolsover complex — the Association of Scientific Technical and Managerial Staffs. The abbreviated report is totally different from the original. In addition to its selective reporting, the union version says there were no statistically significant differences between the dioxin-exposed group and the controls, a statement which is quite untrue.

The most worrying aspect of this affair however, is the position of the HSE. The Executive has said that it is satisfied that the Coalite workers have not been unduly affected by their exposure to dioxin. Yet, if it has never seen the results of the clinical investigations, how can it express such a view? It says that it has to rely on the good faith of the company on this matter. According to an HSE spokesperson, when a product is no longer manufactured the Executive has no legal powers to demand medical records of workers. It is abundantly clear that if the Executive is to do its job properly it should have access to this information and should be given the legal powers to demand it. **Alastair Hay**

Unions want 2,4,5-T ban

THE UK Trades Union Congress has called called for an immediate ban on the use of 2,4,5-T, pending a thorough investigation into its effects by the Health and Safety Executive. The National Union of Agricultural and Allied Workers has already advised members not to handle it. 2,4,5-T is widely used by the Forestry Commission in the UK, which has rejected such alternatives as manual or mechanical clearing as too costly. Two County Councils in England have also banned the pesticide.

The Ministry of Agriculture's Pesticides Advisory Committee has investigated 2,4,5-T eight times, and stuck to its conclusion that it is safe as long as handled in accordance with instructions. The TUC has condemned these enquiries as inadequate. □

United States

Patent law changes opposed in the Senate

ATTEMPTS to liberalise US patent laws by granting universities — and small businesses — exclusive rights to patents from federally-funded research have run into stiff opposition in the Senate on the grounds that they are selling out the American tax-payer.

Many leading research universities have campaigned actively in support of a bill, introduced by Senator Birch Bayh and Senator Robert Dole, which would grant such exclusive rights to any university or small business able to show that it is prepared to spend additional funds on encouraging the exploitation of the patent.

However, when the bill, which has been unanimously approved by the Senate Judiciary Committee, was introduced on the floor of the Senate last month, it ran into unexpectedly vehement opposition from Senator Russell Long, powerful chairman of the Finance Committee and long an opponent of apparent monopoly practices.

Senator Long threatened to filibuster the bill, hinting that this might place in jeopardy the progress of windfall profits legislation on the oil industry. The bill was quickly withdrawn from the debate by Senate leaders, worried that it might otherwise be lost, but is expected to be brought up again shortly.

Meanwhile Senator Long sent a letter to his Senate colleagues last week outlining his opposition to measures which he said represented a "radical and far-reaching giveaway" by the federal government, and would if passed "wipe out every law on the books which reserves for the public the paid results of [federally-financed] research".

Patent law revision has become one of the cornerstones of current attempts both within Congress and by the Carter administration to create an environment considered more conducive to technological innovation by private industry.

The Bayh Bill attracted over 50 co-sponsors when it reached the floor of the Senate. In speaking to the bill, Senator Bayh repeated a common complaint that although the government lacked the resources to develop and market products arising from federal research, it was unwilling to relinquish patent rights in a way that would encourage others to do so.

Similar arguments were heard from others in the debate. Senator Strom Thurmond pointed out that universities had successfully licensed 33% of the patents that they held, whereas 95% of the patents owned by the government had never been exploited. And Senator Edward Kennedy argued that, by helping raise the general level of economic competition, the legislation "would help America retain its

competitive edge in technology and innovation".

To refute charges that the government is selling out investments in research made by the US tax-payer, the bill contains a formula by which funds will be returned to the government from those inventions that eventually enjoy substantial success.

But this has not dampened Senator Long's opposition. In his letter to Senate colleagues he agrees that economic growth and increased productivity "require the most rapid dissemination of scientific and technological knowledge" — but argues

that allowing firms to file private patents would do the opposite by "bottling up" technical information.

In support he quotes Nobel Prize winner Wassily Leontief, Admiral Hyman Rickover, and Michael Pertschuk, Chairman of the Federal Trade Commission, who told a Senate committee in 1977 that there was no basis for claims that giving away title to private contractors promotes commercialisation of government-financed invention, and that the available evidence "shows the opposite". □

Third World research institute loses Congressional approval

THE Carter administration seems to have failed in its attempts to establish a new body to promote scientific and technological research efforts relevant to the needs of developing countries, known as the Institute for Scientific and Technological Cooperation (ISTC).

Delegates from the Senate and the House of Representatives, meeting last week to resolve differences of opinion over foreign aid spending in 1980, agreed to allocate \$12 million to the Agency for International Development (AID) to support programmes of scientific and technical cooperation — but rejected the proposal to establish a new institute to carry out such programmes.

Thus although the institute legally came into existence last October, following agreement between the two legislative bodies on authorising legislation, all of its activities will now be incorporated within AID, with responsibilities that were to have been delegated by Congress to the director of the ISTC now being assumed by the administrator of AID.

The rejection of the ISTC by Congress, which seems inevitable even though the conference report could theoretically be objected to by either House, is a major blow to the administration, particularly since the institute figured prominently in the US presentation to the United Nations Conference on Science and Technology for development in Vienna last August.

From the beginning, administration officials have argued that the new institute, plans for which were first announced by President Carter in a speech in Caracas in 1978, would not be acceptable to Congress if it was run by a totally independent board — an alternative proposal actively canvassed by some scientists — but that a new body operating under the broad umbrella of AID should be acceptable.

Many in Congress have not been

convinced by the arguments for a new institute. In particular, Senator Peter Deconcini last summer persuaded the Senate to reject authorisation plans for the institute on the grounds that it would create unnecessary additional bureaucracy.

This decision was reversed in meetings with the House of Representatives on the authorising legislation. But Senator Deconcini used the same argument last October to persuade the Senate to cut off funds for the institute, and this time his arguments prevailed in conference, although House delegates managed to argue the extra money for the AID budget.

Administration officials are debating whether, now ISTC has been authorised but not funded, they should push ahead for the \$95 million requested for the institute in the 1981 request. But such a move could jeopardise the \$15 million requested for the new Science and Technology Fund being set up by the United Nations Development Programme, which has already been cut back to \$10 million by a House subcommittee.

Those who have been following the changing fortunes of the ISTC in Washington last week expressed disappointment, but little surprise, at the outcome, since the debate over the institute has become bogged down in a political climate increasingly unsympathetic to providing assistance to Third World countries.

However some now feel that the time may be ripe for other initiatives. The National Science Foundation, for example, has recently announced a new programme to encourage US universities to establish links with Third World research groups. Others are suggesting that private foundations might be persuaded to support a scaled-down version of the original ISTC proposal.

David Dickson

International exchange

Hamburg forum a success — of a kind

"It was a success. All twenty speakers at the closing session agreed that it was a success. The fact that everyone stayed and there is a final statement is a sign of success".

Such was the summing up of the executive secretary, Dr Klaus Gottstein to *Nature*, when, after two weeks work and a marathon all-night session that ran on past the official closing date, the Hamburg Scientific Forum managed to hammer out a final statement that was more than diplomatic lip-service to peace and détente.

For in addition to noting that since the signing of the Helsinki Final Act there had been a "significant expansion of scientific cooperation . . . greater in some areas than in others", observing that "different levels of scientific development . . . should be taken into account when planning scientific cooperation", and laying the foundations for convening a further forum in the future, the final statement contained three proposals on subjects which had been the focus of controversy throughout: human rights, the international training of young scientists, and the freedom of scientific exchange and communications.

While there was no reference to Sakharov in the final statement — although the US delegate did raise the issue again in his closing address — the human rights issue did find a place there in general terms.

Increased scientific cooperation, said the statement, can only be achieved "by respect for all the principles and by full implementation of the relevant provisions of the Final Act. All participating states are therefore urged to observe the spirit and the letter of the Final Act, particularly with respect to the conditions essential for scientific cooperation."

"It is furthermore considered necessary to state that respect for human rights and fundamental freedoms by all states represents one of the foundations for a significant improvement of these mutual relations and of international cooperation at all levels."

One subject which the Yugoslav delegate, Dr Drago Ocepek, had earlier associated with human rights was the international training of young scientists. Several delegates had endorsed this theme, including Dr Helge Gyllenberg of Finland and Dr J J Went of the Netherlands. This approach, however, is not popular with the Soviet Union, which tends to view visits abroad as a reward for achievement rather than a stimulus to further effort. Indeed, throughout the two weeks, the Soviet delegates had shown a marked reluctance to discuss any practical details of the logistics of exchange, claiming that the agenda should be confined to professional

discussions on the selected subjects — energy, urbanisation, food production and cancer, cardiovascular and virus diseases.

Nevertheless, international "training courses for young scientists . . . that would enable them to study new science and methods for shorter or longer periods" found a place in the final statement, with the recommendation that "information about these facilities . . . should be disseminated as widely as possible".

The other major barrier to international exchange — restrictions on the free circulation of scientists and information had also produced vigorous confrontations. There were the routine complaints about Soviet scientists who fail to arrive at conferences where they are scheduled to speak — countered,

somewhat irrelevantly, by an accusation from the Soviet Union that in 1976 the University of Geneva had refused to let an Iranian "assistant" in the law department deliver lectures in Marxism. Full freedom of exchange, said the Soviet and East German delegates, was only possible in conditions of complete disarmament.

From the Danish delegation, Dr Maaloe had described the work of the ICSU Committee on the Safeguard of the Pursuit of Science, and stressed the scientific and economic losses caused by unnecessary secrecy. All this was condensed in the final statement to a clause urging "equitable opportunities for scientific research and for wider communications and travel necessary for professional purposes".

Vera Rich

US academy suspends exchanges with Soviet Union

THE council of the National Academy of Sciences has voted to suspend for a period of six months all bilateral symposia, seminars, workshops and new initiatives with the Soviet Academy of Sciences, in protest at the internal exile from Moscow of physicist Dr Andrei Sakharov.

The first meeting to be affected by the academy's decision will be a conference on the interactions between laser beams and matter which was to have taken place at the University of Arizona in early March, and to which 20 Americans and 15 Soviet scientists had been invited.

Three other meetings over the next six months will also be affected, including meetings between scientists from both sides

on basic research, on physics and on experimental psychology. However the council has stressed that its decision is not meant to affect individual contact between US and Soviet scientists, which it says "are matters properly left to the consciences of the participating individuals".

The academy's decision was agreed by a vote of 10 to 3, although some of those who voted against are thought to have pushed for firmer measures, such as extending the suspension of exchanges to one year.

In a telegram sent after the council's meeting to Academician A P Aleksandrov, President of the Soviet Academy of Sciences, Dr Saunders MacLane, Vice-President and acting chairman of the NAS council expresses "our profound hope that the safety and freedom of movement of academician Andrei D Sakharov and his family will be protected".

Dr MacLane also says that the policy adopted by the council — which has been organising exchanges with the Soviet Union for 21 years — reflects the strongly held views of many US scientists. "Our council hopes that the circumstances that have led to the adoption of that policy will soon change so as to permit restoration of the full exchange programme which we have, until recently, viewed with great satisfaction".

The council adds that it sees "no long term national benefit in modifying scientific exchanges to every political action and reaction", but that it is taking action following repeated requests from its members. Dr Sakharov was made a foreign associate of the academy in 1973.

David Dickson



United Kingdom

London medical colleges for the chop

THE Westminster Medical School, and the pre-clinical courses at King's College and the Royal Free Hospital should close, according to a London University working party whose report was published last week. In addition the British Postgraduate Medical Federation would be disbanded and five of its smaller institutes would lose their separate identities. Chaired by Lord Flowers, the committee was charged with rationalising expenditure on medical education in the university while retaining student numbers and staff.

Savings of £6 million a year would result, says the report: £1 million on maintenance of the premises to be vacated; £1 million on administration; £1 million on academic services; and a further £3 million "from the eventual rationalisation of academic departments and academic posts". The present cost of the schools to the university is some £51 million a year. The savings would be redistributed among the remaining medical schools.

In terms of research, the principal casualty of Flower's proposal would probably be King's College — one of only two schools in the university (the other in University College) to operate its pre-clinical teaching on a multi-faculty university campus rather than in a hospital.

"Research is not threatened directly" says Peter Baker, professor of physiology at King's, who estimates that King's receives 24% of all the Medical Research Council and Science Research Council grants for pre-clinical science subjects to the University of London Medical Schools. But if King's loses its medical students, staff members are likely to be reduced and their research with it.



Lord Flowers: finding a way to rationalise expenditure.

Baker feels that the working party has been unduly influenced by a desire to bring pre-clinical teaching within hospitals, in direct opposition to the recommendations of the Royal Commission on Medical Education (the Todd report of 1965) — which stressed the need for a broader-based, multi-faculty organisation for medical teaching.

The battle lines are drawn, Baker believes, between the medical profession which would like to see students exposed to hospital life as early as possible, and the scientists who believe that a medical student requires a thorough grounding in scientific method. Also at stake, says Baker, may be the money involved in pre-clinical education — twice the amount spent on the clinical phase.

When clinical and pre-clinical education are combined 'vertically' within a teaching hospital, says Baker, with the division of funds controlled by hospital committees, "clinicians are very effective in giving the pre-clinicians little money". Pre-clinicians were not represented on the Flowers committee, Baker notes.

The report will be considered by the university for a decision in July. □

High Energy Physics

LEP by 1986?

THE Large Electron-Positron collider, LEP, could produce its first beam by 1986 — three years ahead of the original target, the European Organisation for Subnuclear Physics, CERN, is expected to announce later this week. A Committee of Council meeting last Friday approved this advanced programme, although CERN member states have not yet received a formal proposal.

If CERN members are favourable, there could be a decision within a year to build LEP — while keeping to a 'constant' budget. Pressure from the Italian delegation, plus the prospect of competition from the United States have led to CERN's new sense of urgency.

The attitude of Professor Herwig Schopper, who is likely to be elected CERN's next director-general in June, may also have contributed. Professor Schopper told *Nature* recently that LEP could be built within five years of a positive decision, "but we will have to cut out all the frills". The minimum 'one-sixth' LEP defined in earlier CERN designs could be trimmed, thinks Schopper, to a 'budget-LEP' without changing the size of the tunnel or compromising the chase for the intermediate vector boson. "Building electron accelerators is very different from building proton accelerators" he says. "In our last accelerator at DESY [PETRA] most of our calculations were proved wrong. It will be the same with LEP."

Building LEP will thus be an experiment. Sophisticated orbit correction mechanisms should be built when needed rather than designed *a priori*. Financial savings could also be found in the experimental halls. **Robert Walgate**

ARMS proposes security for 60% of untenured researchers

THE UK Association of Researchers in Medical Science has prepared a document showing that 60% of present contract workers could be placed in tenured career tracks with no additional expenditure and no reduction in the workforce. The plan calls for the creation of a pool of tenured researchers to be funded out of the £81.5 million now used to support contract work by five main sources; the MRC (£24.8 million), the Department of Health and Social Services (£18.3 million), charities and trusts (£22 million), pharmaceutical companies (£11.9 million) and the Royal Society (£4.5 million). ARMS proposes the creation of research institutes based at

universities or medical schools typically employing 85 researchers, 50 of whom would be on tenured tracks and 35 post-doc. The cost per institution would be £1.29 million per year based on an average salary of £9,000 plus 25% for pension and insurance plus £2,500 running expenses plus a 10% institutional surcharge.

Surveys of three industrial laboratories show that total running costs of a research establishment of 2000 employees averages £20 million a year, a figure that compares favourably with ARMS' estimates. The total cost of the ARMS scheme nationally for the estimated 4500 researchers currently employed on short term contracts

would be £68 million. The proposed 10% institutional surcharge would be used to build up an operating fund for tenured researchers out of the estimated £26 million currently supplied by charities and trusts who cannot commit their money on a long term basis.

Representatives from ARMS discussed the proposal last week with Professor James Gowans, Secretary of the Medical Research Council who requested that it be taken to the universities first for their reactions. Gowans told *Nature* that "ARMS is writing to the wrong address. If they get a deal from the universities then I'll have a look at it". **Joe Schwartz**

NEWS IN BRIEF

Spanish science lurches ahead

SPANISH scientists have raced feverishly to meet a 1 March deadline for applications for newly released research funds. Announced in mid-January by the Comisión Asesora de Investigación Científica y Técnica, the amount of 1600 million pesetas (£10.6 million) is the first money made available to researchers since 1977. In addition the commission announced the formation of a committee of 20 to map out future Spanish research strategy. Researchers fear that the committee may impose a straightjacket on the fragile state of basic research out of an inability to realistically assess what kind of research is in "the national interest". A three year plan for restructuring research backed by 50 billion pesetas (£350m) will be tabled in the parliament this Spring.

Canadian research threatened

THE Science Council of Canada has warned that threats to the quality of Canadian higher education, where university enrolment is currently decreasing even though the number of 18-24 year olds is increasing, could have a severe impact on the future strength of Canadian research.

The report* says that for the past ten years, the financial resources for teaching and research provided by both the provinces and the federal government have generally been less than sufficient to keep up with inflation, leading to the serious erosion of research facilities built up in the 1960s.

It says that in addition to seeking more money, universities will have to make a number of hard decisions. These include the elimination of courses and programmes where the number of students and the resources for teaching or research are inadequate for productive scholarship of the highest quality; the transfer of faculty and facilities to other universities; the consolidation of graduate work in specific disciplines into joint centres embracing more than one university; and the common use of major facilities.

**University Research in Jeopardy: The Threat of Declining Enrolment. Available from the Canadian Government Publishing Centre, Supply and Services Canada, Hull, Quebec.*

Pharmaceutical company to rent space at Yale

MILES Laboratories Inc, a subsidiary of the West German chemical and pharmaceutical company Bayer AG, is to rent space in a building allocated to Yale University's department of biology to

establish an institute for pre-clinical pharmacology.

Initially seven or eight scientists and technicians are expected to work on the research and development of new pharmaceuticals, primarily in the cardiovascular field, in a section of Yale's Osborn Memorial Laboratories, which the company has agreed to renovate as part of its payment to the university.

"This agreement is especially significant for the basic biological sciences, since close ties with industry have not existed previously at Yale," Dr Frank R. Ruddle, professor of biology and human genetics, and chairman of the biology department, said in announcing the agreement. "Because of new technical and theoretical developments in biology, we believe that such relationships will be mutually beneficial and therefore more common in the future."

Stanford announces new centre for electronics

THE University of Stanford has announced plans for a new Center for Integrated Systems, bringing together in a single research laboratory individuals with experience in physics, materials science, electrical engineering and computer research to work on very large scale integrated systems (VLSI) for industry and business management.

"Our goal is to make Stanford the premier centre in the world for VLSI research" said Dr James F. Gibbons, professor of electrical engineering, in announcing plans for the \$15 million centre.

The three goals of the new centre are: to produce approximately 100 MSc and 300 PhD graduates per year ("tomorrow's technological leaders" according to Professor James Meindl); to promote research, including the development of working design automation capability and computer-managed fabrication facilities; and to offer, via instructional television and videotapes, new courses, conference and workshops "designed to keep scientists and engineers now employed in industry abreast of rapid developments in the field." Funding will come principally from government and industry sources.

Fusion beam injection success

SCIENTISTS at the University of California's Lawrence Berkeley Laboratory last week reported that they have successfully tested a neutral beam injection system which will be used to heat the plasma of the Tokamak Fusion Test Reactor at Princeton Plasma Physics Laboratory, due to start operating in December 1981.

According to Dr Kenow H. Lou, project manager for the injection system at LBL, deuterium beams were successfully accelerated to 120,000 electron-volts, producing seven million watts of power in pulses lasting 0.5 seconds. The team is now studying ways of increasing the duration of the beam to 1.5 seconds.

The Princeton Tokamak will use four such neutral beam injection systems, the prototype of which has been built as a collaborative effort between LBL and the Lawrence Livermore Laboratory, which carried out the beam line engineering. Following the successful tests — described as a "major milestone" in the US fusion energy programme — the prototype system will remain in operation to develop further information and experience which may affect TFTR operations.

UK short of basic grade nuclear inspectors

THE Nuclear Installations Inspectorate is 53% below its full complement of basic grade inspectors according to information released last week by the Department of Employment. In answer to a parliamentary question by Frank Hooley (Labour, Sheffield) the department revealed that the NII was having difficulty recruiting structural, electrical, mechanical and instrumentation engineers and has a shortfall of 14 out of its complement of 26 positions. A Health and Safety Executive official told *Nature* that routine work was not affected but that in the review of the pressurised water reactor specific areas of work might have to be subcontracted to the Safety and Reliability Board of the UK Atomic Energy Authority. The 61 principal inspectors and 26 inspectors of the NII are responsible for surveillance of 11 nuclear power stations, three radio-chemical centres, five nuclear fuel production works and the UKAEA's facilities at Windscale, Dounreay, Springfield, Winfrith, and Harwell.

CERN opens data transmission experiment

THE European STELLA experiment to transmit scientific data by satellite between sub-nuclear physics laboratories was initiated today in Geneva. Directors-General of the European centre for nuclear research, CERN, the Internal Market and Industrial Affairs Commission of the European Communities and the European Space Agency took part in a ceremony inaugurating fast and reliable data transmission between CERN and laboratories in Hamburg, Saclay, Oxford, Pisa, Dublin and Graz. The STELLA system will transmit data at 1 megabit per second using the European telecommunications satellite OTS-2.

No technical fix to a political problem

THE International Nuclear Fuel Cycle Evaluation ended on 29 February with a plenary session that adopted the reports of all the study groups. **Hermann Bondi** (right) Chief Scientist at the UK Department of Energy and leader of the British delegation to INFCE, assesses its work



THE INFCE reports naturally reflect many points of view, yet they were unanimously agreed. Some countries had suggested 'technical fixes'; some claimed that energy independence was necessary, and that the whole argument about non-proliferation was just a device to keep a vital new technology firmly confined to the most advanced states. But it was agreed that there is no technical fix to the political problem of proliferation. There is no fuel cycle that presents no such risks and dangers. On the other hand the working groups looked closely at the sensitive areas; how they could be made less risky, what could be done to improve their security, and what institutional measures could be evolved which would further increase international re-assurance.

I concentrate on four areas: the availability of nuclear fuel, the economics of the fuel cycles, the risks of proliferation, and the terms and conditions of nuclear supply agreements. Safety and environmental questions were not addressed in any great detail.

INFCE assumes a fairly high rate of growth in nuclear generating capacity. Compared with about 125 GW of capacity today, INFCE projects 850 to 1200 GW in

the non-Communist world in 2000, and 1800 to 3900 GW in 2025. With use of the once-through cycle and on its high projection of growth, INFCE concludes that the lifetime uranium requirements of the reactors installed and in operation by 2000 will approach current estimates of known uranium resources (5 mTU). These figures have been criticised, notably in the US, for being unduly pessimistic about uranium availability.

But the figures are not everything. INFCE rightly stressed that the uncertainties in these forecasts are great — not just in the prediction of nuclear demand and of the extent and accessibility of geological reserves of uranium. The uncertainties in the political availability of uranium are greater perhaps than for any other commodity. The message is simple. If countries are to be confident that there will be enough uranium to fuel their reactors over the next 30 to 40 years, acceptable solutions to political and environmental problems limiting the exploitation of uranium must be found, new sources of uranium must be discovered and brought into production, and reactor strategies that conserve uranium must be adopted by at least some of the big users.

The scope for new discoveries of uranium is good. Estimates range from 6.6 to 14.8 mTU. If these resources can be discovered, brought into production, and the uranium made available internationally, supply should meet the requirements of INFCE's perhaps more realistic low projection of nuclear growth to beyond 2025. But the extent to which this will happen is uncertain. The more pessimistic a country, and the more dependent it is on uranium imports, the more likely it is to want to move to the early introduction of reactors that conserve uranium, such as advanced thermal reactors and fast reactors.

Fast reactors offer the prospect of nuclear development eventually almost free from uranium supply constraints. So while INFCE does not say that the early introduction of fast reactors is essential on resource grounds, it does illustrate the impact of decisions about reactor strategy — and their timing — on uranium supply and demand. Such decisions, like many in energy policy, depend largely on a country's perception of its need to be

insured against uncertainties in fuel supplies.

In discussions on the economic aspects of the fuel cycles it became clear that considerations vary from country to country. No fuel cycle can be said to have a universal economic advantage over the next few decades. Recycle into light water reactors is of only minor economic advantage, but may offer the benefits of reducing reliance on imported uranium. On the other hand, if the capital and fuel cycle costs of fast reactors can be brought down, fast reactor recycle could offer considerable advantages.

INFCE came to no definite conclusion on when the fast reactor would compete economically with thermal reactors. The uncertainties in the future price of uranium and of fast reactors, and regional variations in uranium supply, mean that countries will take different views about when to proceed from thermal to fast reactors.

Much of INFCE's discussion centred on two related subjects: how the risk and the fear of weapons proliferation can be reduced, and how to make nuclear energy widely and assuredly available.

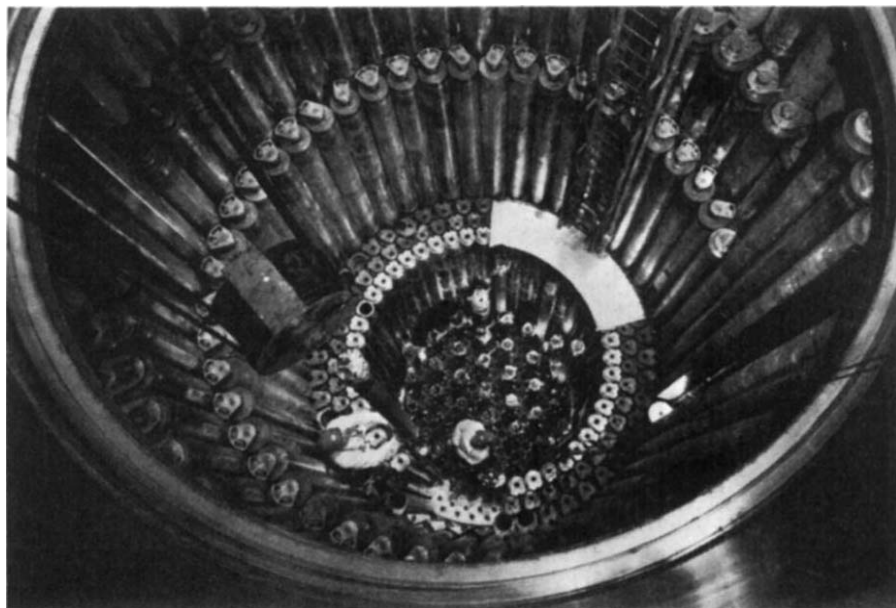
From the start it was recognised that a decision to construct nuclear weapons is political, and that the misuse of the civil fuel cycle is not the easiest or cheapest route to acquiring them. INFCE was a technical, not a political evaluation. The reports, therefore, look only at the way in which a civil nuclear programme might help a country make a nuclear explosive.

The misuse of fuel cycles that involve the handling of large quantities of weapons-usable material — not just plutonium, but highly enriched uranium (HEU) or uranium 233 — might offer a path. But discussions in the Working Groups and in the TCC showed that comparisons between the proliferation risks of the various fuel cycles were extremely complex. How, for example, does one assess the relative proliferation

Background and participants

INFCE was set up in Washington in October 1977, in response to heated disagreements between states over the nuclear fuel and technology trade. In particular, President Carter had aroused anxiety among developing nations in April that year when he called a halt to the separation and use of plutonium, implying the deferral of reprocessing and confinement to a once-through cycle.

Sixty-six countries took part in the discussions; industrialised and developing; weapons and non-weapons states; East and West; nuclear suppliers and customers; those that had signed the Non-Proliferation Treaty and those that had not. Five international organisations also took part. The Evaluation was not, however, a negotiation. The Organising Conference set up eight working groups, linked by a Technical Co-ordinating Committee.



Refuelling the UK's Prototype Fast Reactor at Dounreay, Scotland. Facilitating supply of this fuel (plutonium oxide) without risking weapons proliferation was one of INFCE's major concerns

risks of the various fuel cycles were extremely complex. How, for example, does one assess the relative proliferation risk of an enrichment plant, which produces HEU for a research reactor, with that presented by spent fuel (which necessarily contains plutonium) stored in ponds where it becomes increasingly accessible as its radioactivity decreases?

INFCE concludes that the proliferation risks of fuel cycles cannot be compared in the abstract, but depend on the specific arrangements. No single judgement about the relative risks of proliferation can be made which will be universally and permanently valid. The reports look at those fuel cycle activities that are sensitive and concentrate on measures to reduce the risks of proliferation arising from them.

INFCE examines the various technical options for reducing the proliferation risks of the back end of the fuel cycle. Measures to reduce the presence of weapons-usable material in separated form in the fuel cycle, such as the co-conversion of the uranium and plutonium solutions produced at reprocessing plants directly into mixed oxide; measures to use radioactivity to protect these materials from diversion, for instance, by spiking fuel with high energy gamma emitters; and measures to protect them by the use of physical barriers, such as providing extra containment around reprocessing plants.

Measures using radioactivity as a protective barrier not only involve considerable environmental and economic penalties, but also make safeguards more difficult because of the material accountancy problems created by the intense radiation. The other measures, though perhaps desirable for future plants, turn out by themselves to have only a limited influence on proliferation risks.

INFCE thus could not identify any

potential technical fixes in this field, but stressed that research reactors could and should be converted to require only uranium of low enrichment. This was the only useful technical measure emerging from a full discussion of the proliferation risks arising so seriously in enrichment.

More promising are institutional measures. Multinational fuel cycle ventures can offer a country seeking fuel cycle services a more economic way of meeting their needs, and are a potentially useful measure for reducing proliferation risks. INFCE points that a first step towards such institutional arrangements would be for countries with large fuel cycle facilities to offer services to others. This would help postpone the need for the latter to build their own fuel cycle facilities.

But perhaps the most promising institutional measure is a scheme for international plutonium storage so that plutonium was only stored under international supervision and released solely for safeguarded peaceful uses. This could have important non-proliferation advantages and INFCE applauds the work being done in the International Atomic Energy Agency to establish such a scheme.

The central feature of the non-proliferation regime, however, is the network of agreements, generally implemented by the IAEA by which states accept the international system of inspection or "safeguards" on their nuclear activities. INFCE sees no problems with the techniques applied to existing operating plants, but foresees an important need for development work on the techniques for safeguarding plants handling large quantities of weapons-usable material. A crucial point is the need for security objectives to be considered at the design stage of any plant.

One thing, however, that INFCE does

not go into is how to win wider acceptance of safeguards, mainly because this is a political question not in INFCE's remit. But there is an area of INFCE's work which may provide part of the answer. Working Group 3 was devoted to the question of how to improve "assurances of supply" — assurances that once a nuclear supply contract has been signed, neither government will interfere in its performance. In recent years some suppliers, notably the US and Canada, because of growing worries about proliferation, have cut off previously agreed supplies. This has reduced the credibility of such arrangements, and has tended to make customers more determined to make themselves self-sufficient in nuclear supplies and services in the long run.

Working Group 3 has looked at the way in which suppliers can be given confidence that their non-proliferation concerns can be met, while at the same time giving customers the confidence that their supplies will not be interrupted. This is the central theme of non-proliferation policy, and the strength of feeling among the developing and other customer countries about their need for nuclear energy makes its solution of crucial importance. Working Group 3 suggests that a consensus on the non-proliferation conditions of supply could first be expressed in "common approaches" to bilateral agreements, which might later evolve into a more formal multilateral agreement on the terms and conditions of nuclear trade. These ideas should be followed up, and the IAEA offers an ideal forum for doing so.

The findings of INFCE do not reflect only the views of a few large industrialised nuclear countries. The Evaluation has respected the views of the countries with small nuclear programmes, the customer and developing countries.

Looking at the resource arguments, it describes the reasons why countries wish to keep open the option of plutonium recycle. Looking at the economic arguments, it shows why many countries look ahead to fast reactors so as to extract the maximum energy value from limited uranium resources. Looking at non-proliferation questions, it shows how pointing accusing fingers at different fuel cycles or seeking alternatives can uselessly divert attention from the more fruitful task of improving safeguards and creating suitable institutions. And finally, it suggests how work towards an improved consensus on the conditions of nuclear trade would both promote the aims of non-proliferation and help ensure that nuclear energy is available to meet the world's energy requirements.

It is a triumph of common sense and of common purpose that the very wide cross section of countries represented in INFCE, created by the US initiative, could arrive at this consensus, which while in no way underwriting the original American specific measures, points a way forward. □

Europe embraces the fast breeder reactor

The International Nuclear Fuel Cycle Evaluation has given a boost to the fast breeder reactor. But while the US is rejecting the breeder, others are embracing it, writes **Robert Walgate**

FRANCE'S fast breeder programme is already many years ahead of any other nation's. Last week Electricité de France announced an order for a further two fast reactors of 1500 MW each to supplement Super-Phénix, a commercial demonstration reactor due to produce electricity in 1983, so confirming the French lead.

But while France may be ahead in practice, other European countries are with her in spirit. Last month the Council of Foreign ministers of the nine-nation European Community drafted a resolution urging member states which have fast breeder programmes to "get on with them", and offering "appropriate" EEC support. Already, Euratom loans are available to countries developing fast breeders.

Moreover, the Assembly of the Council of Europe, a loose association of 18 European states, recently adopted a resolution that "current programmes in Europe for the development of fast breeder technology to the point of commercial-sized demonstration plants should be continued".

Dr Walter Marshall, Deputy Chairman of the United Kingdom Atomic Energy Authority, and a prominent member of INFCE, argued last week that countries with an advanced nuclear programme, such as those of Europe, should concentrate on fast breeders, leaving

thermal reactors to other countries.

Dr Marshall, who once described INFCE as "an exercise in proliferation" because it would help communicate sensitive technologies from the most to the least nuclear of its 66 participants, said that such a regime "would not guarantee the non-proliferation of nuclear weapons . . . but it does give the maximum opportunity to governments . . . to secure the most proliferation-resistant regime possible."

Last week the UK House of Lords debated the fast breeder issue, prompted by the Council of Europe resolution. Viscount Thurso, whose fief includes the Dounreay prototype fast reactor, compared progress.

"Our own PFR deliberately contains fuel elements of commercial size such as will be used in a commercial demonstration fast reactor, and employs boiler and coolant pump designs of a commercial sort which will only have to be scaled up slightly for CDFR. Super-Phénix will have to use fuel elements and boilers of designs hitherto untested in practice, and will in consequence have a fairly large question mark hanging over it."

In the US "EBR-II is still running but FFTF is doing criticality tests only and work on CFBR has stopped . . . The state of progress in Russia indicates that BN600 is not yet critical and may not be operational until 1983 and although they are preparing to start on BN1600, they have a long way to go with its design, especially in the field of fuel technology."

In Japan "where they very much need atomic energy . . . they are very far behind schedule. Monju, scheduled for operation in 1986, is not yet in building and it is only the equivalent of our PFR."

"In Germany, the KNK11, is only a converted thermal reactor and SNR300 is delayed by legislation; SNR2 is only a gleam in their eye. In France alone is progress being maintained, although in my view they are behind us is fuel element and boiler design and have an unproved fuel cycle."

Britain should thus move rapidly to the promised public enquiry on the CDFR, argued the Viscount, and in this he was supported by most speakers. Only two strong voices were raised against — those of Lord Avebury, an engineer and a Liberal, and Lord Bowden, an ex-Secretary of State for Education and Science.

Lord Avebury welcomed the debate not because of the immediate need for the fast breeder but "as a warning of the powerful onslaught which is to be expected from the

nuclear lobby if, and when, the government initiate the public inquiry".

"I think that the adoption of breeder technology, plus the reprocessing of the spent fuel from thermal reactors, which of course involves the separation of plutonium, would mean that by the middle of the next century . . . there would be 25 or 30 nations with at least a primitive weapons capability."

And Lord Bowden questioned the economics of the reactors. "No-one can be found to deny that a fast breeder reactor will cost very much more than a conventional type. The ratio has been variously estimated at five times. The Germans have found that their own estimated cost has escalated ten times since they first started on the work."

The price of uranium must rise dramatically, said Lord Avebury, if the fast reactor was to be economic — perhaps from its present level of \$40 per pound to \$218 per pound, according to a study prepared by the US Arms Control and Disarmament Agency. But at these prices, argued Lord Bowden, there would be an increase in the size of economic uranium by a substantial factor, perhaps sufficient to equal the maximum breeding factor attainable in a fast reactor.

This breeding factor (the energy obtainable per pound of uranium in a fast breeder as compared with a thermal reactor cycle) "will be less than a dozen times in practice on any system which has so far been proposed and worked out in detail" and not the theoretical factor of 70 commonly quoted. "The Americans are very shrewd in their assessment of economic potential" said Lord Bowden "and they have decided there is no case for developing the fast breeder for several years to come . . . So I am afraid that if we go ahead with the fast breeder in Europe we shall repeat almost precisely the story of Concorde: it will be a technical triumph and an economic catastrophe."

Lord Bowden urged the government instead to consider the Canadian CANDU reactors "which get three of four times as much power per tonne of uranium" as pressurised water or advanced gas-cooled reactors as a way of conserving uranium. "The amount of power we shall in practice get from fast breeders is nowhere near the theoretical amount postulated. It will almost certainly take at least 20 years before the fast breeder can double the amount of fissile material it has to start with. It will take several tonnes of plutonium to start off a fast breeder and, when it has been started, the plutonium and the blanket have to be taken out and reprocessed chemically many, many times."

"I beg the government" said Lord Bowden "to consider this as an alternative". □

● Maurice Bazin reports from Rio de Janeiro on the Brazilian response to INFCE.

The response is one of relief and diplomatic excitement. The controversial multi-billion dollar nuclear agreement with West Germany, signed in 1975, which envisages the construction of up to eight plants of 1,200 MW by 1990, accompanied by uranium enrichment and fuel reprocessing facilities, remains unaffected.

"There was a consensus at the meeting that its conclusions will have no moratorium effect on our nuclear programme and will not serve as a pretext for modifying existing treaties" declared Hervasio de Carvalho, director of the National Commission for Nuclear Energy (CNEN) who attended the INFCE sessions. "The results will be distributed among all countries without discrimination, but no country will be forced to take special measures as a result of the technical exercises carried out by INFCE."

CORRESPONDENCE

Dioxin, an emotive word

SIR, — The tragic murder of the production director of the trichlorophenol plant in Séveso demonstrates most dramatically that "dioxin" has joined a select group of words including "cancer", "nuclear" and "fluoride" which inspire fear and trigger emotions that require the explanations of psychologists not a chemist.

The ease with which traditional standards of rigour in proof can be abandoned when discussing toxicity should be a warning to all serious scientists. The article by David Dickson (31 January, page 418) can do nothing but reinforce the almost mediaeval atmosphere of mystic suspicion which seems to have enveloped this subject.

Pentachlorophenol is probably not one of the most widespread pesticidal chemicals in domestic use in the United Kingdom, other chlorophenols used in disinfectants could perhaps claim that honour, but it is quite widely used as a wood preservative and particularly in the United States. It has been used for more than forty years, and long before the present emphasis on worker safety, so that a very large number of workers have been exposed to considerably greater dosages than any relevant to the "cases" quoted in the article. There is no suggestion that the handling of pentachlorophenol-based wood preservatives, nor even pentachlorophenol itself, has been a major health hazard to operatives in the industry.

Surely if a material utilised at extremely low concentrations in contact with a consumer were to give rise to such dramatic hazards, then we could look for a large number of fatalities or significant debility in workers in the industry. This is simply not the case.

It is important to stress that the most toxic dioxin — tetrachlorodibenz-p-dioxin, has never been found in industrial pentachlorophenol (of modern manufacture) and the accuracy of analysis is now at a confidence level of one part in ten million. Octachlorodibenz-p-dioxin, which is a known contaminant, is less toxic than pentachlorophenol itself. To claim that little is known of the physical and chemical toxicological properties of other dioxins is unfair, and perhaps the interested reader should refer to "Pentachlorophenol Chemistry, Pharmacology and Environmental Toxicology", editor K. Tanga Rao, "Chlordioxins — Origins and Fate" by Ethyl H. Blair, and "Dioxin — Toxicological and Chemical Aspects", edited by Flaminio Cattabeni, Aldo Cavaliaro, Giovanni Gallis. The hexachlorodioxins, which are found in technical pentachlorophenol of the quality now available in Europe, are at a level of about five parts per million, and the most toxic isomers are at proportionately lower levels. Their chronic toxicity is well established and it would be necessary to take more than the lethal dose of technical pentachlorophenol regularly in order to run the risk of future development of cancer. In effect the acute toxicity of pentachlorophenol protects against long term hazard.

The approach of certain environmentalists and American attorneys seems to centre on contaminants, even if present in extremely small quantities overlooking the accepted acute toxicity of the main ingredients. Furthermore they seem to believe that pentachlorophenol is applied in isolation and disregard co-solvents, solvents, carriers, resins, pigments and dyes, all of which form part of preservative formulations, the majority of which will certainly not have been

investigated for impurities nor their toxicology determined with anything approaching the thoroughness applied to pentachlorophenol. Nor is any mention made of the potential hazards to man of the organisms which pentachlorophenol controls.

Your readers can be assured that there is no iceberg, the divers have been down these many years and would have found it. Can I plead that we remember that all chemicals are toxic, it is only a matter of determining the dose?

Finally, I revert to my opening remark, can any psychologist offer an explanation of this fear and the profound emotions it releases?

Yours faithfully,

J. DAVID

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World energy needs of the future

SIR, — R.B. Temple, in a letter (7 February, page 520) virtually as long as my (necessarily limited) review of *World Energy — Looking Ahead to 2020* (15 November, page 344), says many things I would readily endorse.

It is for the reader to judge whether I was sufficiently critical of the energy strategy proposed by the Conservation Commission of the World Energy Conference. My emphasising, through direct quotation as a form of shorthand, of the Commission's conclusion that their assessment required "doubling nuclear power plant capacity every six years", was indeed meant to signal the reader to the far-fetched numbers this implied and which Dr Temple has calculated. No disagreement here, whatsoever, especially as the latest official UK estimates of new reactor capital costs are nearer £1000/kW, or £1 × 10⁹ for a 1000 MWe plant.

As for my own position in the energy debate, I would suggest an examination of Chapter 5 of *World Futures — The Great Debate* (ed C. Freeman and M. Jahoda), Martin Robertson, Oxford, 1978 which was reviewed by Lord Ashby in *Nature*, (November 9, 1978, page 144). I suspect Dr Temple and I would share much common ground.

Yours faithfully,

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Nuclear energy and the world economy

SIR, — R.B. Temple, who wrote a letter in your 7 February issue (page 520) commenting on the problem of feeding world economy with nuclear energy has been trapped in a famous pitfall, which is to assume that the size of a certain machine, in the particular case that of a nuclear reactor, is God given and time invariant.

Now, if we analyse energy systems or transportation, or chemical ones, we find that there exists a very tight link between the size of a plant and that of the market, in most cases the link being that of simple proportionality. So, if one increases electricity consumption by say a factor of 1000, which happened for example in the US from the beginning of the century to present, the number of power

stations stays constant (or more precisely decreased somewhat) because the stations' size grew with the market. There are presently about 3000 electric power stations in the world, of a certain size and connected to grids, and presumably a smaller number will be present in the year 2020. Consequently the number of plants to be constructed every year if we assume a life of 30 years will be 3000:30 or 100 per year at regime, which is not an extraordinary figure if we think it spread over the world. If we choose an energy vector different from electricity to carry around the energy, the number of reactors will depend on the cost of transportation. If we take hydrogen as an example, transportation costs in the proper context are an order of magnitude lower, and this makes generation possible in fewer and larger machines — say 100 for the whole world, with a replacement ratio of 3 per year. Does this still sound too many?

I am not entering into the complex question of financing energy systems development. I am just attracting attention to the fact that final consumers now pay for their energy something in the range of \$1.5 × 10¹²/year.

Yours faithfully,

C. MARCHETTI

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Fast reactor safety and the fire at Beloyarsk

SIR, — Vera Rich's piece (31 January, page 420) based on a clandestine report of a fire at Beloyarsk in the USSR associates the fire with the fast reactor (BN600), at that site, and implies faults in the safety arrangements for that and, by implication, other fast reactors.

Beloyarsk is the site of two graphite moderated steam-cooled reactors, which were completed some years ago, and of the fast reactor BN600 which, at the time of the alleged fire, was still at least 12 months short of completion. The assumption that the fire, if any, might have led to the escape of radioactive sodium from the primary circuit of this reactor is, therefore, questionable, to say the least.

BN600 is a pool type reactor in which the core, pumps and primary heat exchangers are submerged in sodium contained in a strong double-skinned reactor tank. There is, therefore, no circuit, in the physical sense, which could 'rupture' and 'cause an explosion scattering radioactive material in a cloud . . .'. Vera Rich's reference to 'the 1957 Kyshtym disaster' is equally inappropriate in the light of our ignorance of the nature, cause, or extent, of this incident.

It is not clear what implication the reader is meant to draw from the remark that "the fire at the Shevchenko fast breeder . . ." was not technically a "nuclear disaster". This topic has previously been ventilated in your columns and information has been supplied which demonstrates both that this fire was no sort of disaster and that no radioactivity was involved since the circuit which leaked was a secondary sodium circuit external to the reactor.

Fast reactor safety is of public importance and, in your columns at least, deserves a more objective treatment than is displayed in Vera Rich's article.

Yours faithfully,

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NEWS AND VIEWS

The emergence of modern man

from Gail Kennedy

THE emergence of our own species remains one of the most elusive problems in palaeo-anthropology. Our increasing knowledge about very early hominids, such as the australopithecines, only serves to emphasise our lack of understanding of our own more immediate origins. In recent years, however, a few specimens have been found which give us tantalising views of the emergence of *Homo sapiens* from *Homo erectus*, the presumably ancestral species. One such discovery is reported in this issue (page 55). This well-preserved skull, Laetoli Hominid 18, was recovered in 1976 from the Ngaloba Beds at Laetoli, Tanzania. These beds are in the same area and stratigraphically overlie the Laetoli Beds in which Mary Leakey has found the fossil remains and footprints of early australopithecines.

L.H. 18, dating to about 120,000 years ago, shows a combination of advanced and primitive features. The overall expansion of the skull seen in the vertical parietals, rounded occiput and cranial capacity (1200 cm³) places it nearer modern sapient than to earlier *H. erectus*. In contrast to these more modern features, the large brow ridges, low, sloping frontal, marked bone thickness and certain features of the cranial base place L.H. 18 nearer to the ancestral group. Similar combinations of primitive and advanced characters are seen in other 'archaic' sapient from eastern and southern Africa such as the Omo skulls from the Kibish Beds in Ethiopia and the Broken Hill (Kabwe) skull from Zambia. The dates for these specimens are not entirely satisfactory but the Omo skulls may date to about 130,000 years ago (Butzer *et al.* *Quaternaria* 11, 15; 1969) and Broken Hill to about 110,000 years ago (Bada *et al.* *Proc. natn. Acad. Sci. U.S.A.* 71, 914; 1974). Even less securely dated but

also somewhat morphologically intermediate between *H. erectus* and *H. sapiens* are skulls from Saldanha in South Africa, Bodo in Ethiopia and Ndutu in Tanzania. As a group, these skulls are very heterogeneous but it is undeniable that they demonstrate varying degrees of sapientisation of the underlying *H. erectus* template. Skulls like Bodo and Ndutu, for example, occupy the more primitive *H. erectus* end of the morphological spectrum while others, such as L.H. 18 and Omo differ in fewer ways from modern *Homo sapiens*. Combined primitive and advanced characters are also seen in certain elements of the post-cranial skeleton such as the femora from the Broken Hill locality and from the Guomde Formation east of Lake Turkana (KNM-ER 999) (Kennedy, in preparation). This latter specimen may date to about 100,000 years ago (Fitch *et al.* *Nature* 252, 213; 1974).

The recognition that populations demonstrating a combination of *H. erectus* and *H. sapiens* features existed in Africa before 100,000 years ago poses some very intriguing questions. One such question concerns the source and nature of the evolutionary stimuli which led to the emergence of the modern species. Throughout its long evolutionary history, *H. erectus* demonstrated very little, if any, evidence of progressive morphological change. The earliest presently recognised members of the species, from the Lake Turkana area of Kenya and from Java, predate 1.5 million years. From this time through the Middle Pleistocene there is no clear evidence of progressive morphological advancement. According to some workers the first evidence of sapientisation occurred 300,000 to 400,000 years ago, during European Second Interglacial times. Others however, would maintain that the transition did not occur until about 100,000 years ago, during the Third Interglacial period. The group of archaic

hominids under discussion here, including L.H. 18, Omo and Broken Hill, may add slightly greater weight to a later transitional phase.

In any case, the long period of morphological stasis in *H. erectus* was ended by the appearance of individuals with significantly expanded, less angular skulls and thinner bone structure. It is of great interest that these more advanced morphological characters are not accompanied by obvious advances in stone tool technology, subsistence efficiency, settlement size or duration of occupation. This suite of traits is conventionally thought not to occur until the Upper Palaeolithic, around 35,000 years ago. Moreover, geographic range, already broad in *H. erectus* does not appear to change in the initial phases of sapientisation. What factors then were responsible for the evolutionary pressures and responses which ultimately led to the replacement of *H. erectus* by *H. sapiens*? Although the answer, in a very general way, may rest on increasing complexity of the behavioural repertoire and social organisation a more precise delineation of the process has not yet been accomplished.

A second question concerns the relationship of the very distinctive Neandertals both to these archaic sapient and to modern man. Controversies have continued for many years over whether anatomically modern man rapidly emerged from a single Neandertal/early sapient lineage in the Fourth Glacial or whether Neandertal and sapient lines diverged separately from a common ancestor sometime in the Middle Pleistocene. This hypothesis would view the two lines in coexistence until the extinction or assimilation of the Neandertals between 20,000 and 30,000 years ago. The Neandertal complex of characters first becomes visible in the fossil record in Europe during the Third Interglacial and is

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found widely distributed in Europe and southwest Asia during the early part of the Fourth Glacial. If the dates for the archaic sapients, such as L.H. 18, Omo and Broken Hill are accurate then this group precedes the first known appearance of the Neandertals by a very short period of time. However, the highly distinctive suite of features which characterises the Neandertals is not present in the preserved remains of the archaic sapient group. Absent in the latter group are such Neandertal features as the occipital 'chignon', rounder (as opposed to vertical) parietals, very large cranial capacity and curved, robust long bones. Thus, whatever

the nature and origin of the Neandertal complex of characters, much of this morphology is not reflected in the archaic sapients which immediately pre-date them. From this evidence, can it be argued that a single lineage, anatomically progressive 110,000 to 130,000 years ago, rapidly evolved into the Neandertal populations (which are primitive in many features), then just as rapidly, later evolved into anatomically modern man? On the basis of the available evidence now emerging in Africa a dual lineage, extending back more than 100,000 years, seems a more likely hypothesis. Further confirmation from the fossil record, however, is required. □

Gyromagnetisation: rocks in a spin

from R.L. Wilson

THE fascinating phenomenon of rotational remanent magnetisation (RRM) was unexpectedly discovered some 15 years ago as a result of geophysicists' attempts to interpret the natural permanent magnetisation of their rock samples. In the process of trying to separate the various magnetisations acquired by the rock during its lifetime by demagnetising the rock in an alternating magnetic field, it was found that slow rotation of a sample during alternating field demagnetisation could induce a remanence in the sample along the rotation axis even when no steady (for example geomagnetic) field was present. This phenomenon remains largely unexplained but several attempts at partial explanation are now emerging spurred on by novel and ingeniously derived experimental results.

The serendipitous discovery of RRM followed the use of a method of alternating field demagnetisation devised by Brynjolfsson and Sigurgeirsson at the University of Iceland (*Adv. in Phys.* 6, 247; 1957) to overcome a problem found in early attempts to demagnetise rocks by the application of decreasing alternating fields. It had been found that far from totally demagnetising the rock one actually replaced the original magnetisation with a new one due to the combination of the alternating field and the geomagnetic steady field in the laboratory.

One way of avoiding this was to cancel the local geomagnetic field in some way (As & Zijdeveld *Geophys. J.* 1, 308; 1958) but another was to 'tumble' the specimen to randomise the magnetisation due to the combined alternating and steady (geomagnetic) fields. Brynjolfsson and Sigurgeirsson developed a very clever method which involved rotating the sample

about a single axis defined by its direction of natural magnetisation and applying the demagnetising fields perpendicular to the rotation axis of the sample. As the two magnetisation components had different properties, the total natural magnetisation vector sometimes rotated through quite large angles during demagnetisation, and so the sample was at each stage reoriented in the apparatus to maintain the new direction of natural remanence aligned with the axis of sample rotation.

When we duplicated this apparatus in Liverpool, deciding (for some reason which no-one can now remember) that every other demagnetisation process would take place with the sample turned upside-down with respect to the rotation axis, we obtained to our astonishment field demagnetisation curves which clearly zigzagged about the usual smooth demagnetisation curve. The specimens were demagnetised less when oriented one way up than the other. The first observations of this effect had been published in 1967 by Doell and Cox (*Developments in Solid Earth Geophysics 3; Methods in Palaeomagnetism*, Elsevier, 1967) but they had used three-axis tumblers and their results were much more complicated and were not pursued. Eventually the idea of RRM was arrived at (Wilson & Lomax *Geophys. J.* 30, 295; 1972).

Once this phenomenon had been accepted the problem was to explain it and some recent experimental results are throwing light on this problem.

Interesting experiments by J. Edwards at Aberdeen University (*Geophys. J.* in the press) show that one can induce larger rotational remanent magnetisations by rotating the sample during only part of the demagnetisation process. He also shows that the slow rotation affects magnetic grains whose coercive forces are

considerably greater than the greatest alternating (peak) field applied. This seems to mean that the act of rotation, or 'tumbling', may considerably enhance the effect of a given peak alternating field, in demagnetising a given coercivity range within a sample. Effectively it makes the tumbling method a more powerful demagnetisation method than the stationary one.

Meanwhile, in this issue of *Nature* (page 48) A. Stephenson, of the University of Newcastle-upon-Tyne, reports his direct observations of the torque developed in a specimen which is rotating about an axis at right angles to an alternating field axis. The torque is quite appreciable, and is clearly related to the acquisition of rotational remanent magnetisation. Stephenson uses these results in support of a theory which relates rotational remanent magnetisation right back to the Barnett gyromagnetic effect; where a rock, rotating in the absence of a magnetic field, acquires a magnetisation caused by the alignment of internal magnetic moments along the axis of rotation (*Rev. Mod. Phys.* 7, 129; 1935).

It may well be that rotational remanent magnetisation is providing us with a means to investigate the Barnett effect, which has hitherto been very weak and difficult to deal with. According to Stephenson, the reason it has such a large effect in these experiments is that we are dealing not with the rate of rotation of the whole rock sample, but with the rate of rotation of the magnetic moment of a single magnetic grain when it is flipping between stable states. Since flip times are very brief, such rotational rates can be equivalent to 3×10^7 rotations per second greater. One could never hope to rotate any bulk material in the laboratory at such a speed, to enhance the Barnett effect.

However, that is not the end of the story. A second paper in this issue (page 49), again by Alan Stephenson, shows that curiously enough one does not even need the rotation of the specimen to produce what Stephenson terms a 'gyromagnetic remanent magnetisation'. He shows that one can achieve a similar magnetisation whenever one sense of flip predominates during alternating field demagnetisation, and that this will be observable whenever the magnetic material is sufficiently anisotropic. He has, with considerable ingenuity, manufactured an anisotropic specimen from stacked strips of magnetic recording tape. Sure enough, he is able, with no specimen rotation during an alternating field 'demagnetisation', to induce a remanence into the specimen which is clearly related to its anisotropy and which confirms his suggestion that gyromagnetism is the basis of the whole effect.

Stephenson's and Edwards' experiments are dealing with an effect which will touch upon several kinds of research. First, rotational remanent magnetisation provides us with a fairly easy means of studying gyromagnetism itself. At least

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experimentally there is little difficulty in reproducing the effect, but the theoretical interpretation of the results may be fairly difficult. Second, gyroremanent magnetisation may help us to measure and study magnetic anisotropy from the point of view of the solid state physicist. Third, the measurement of magnetic anisotropy in rock samples may be carried out by this new method, and used to interpret the stress histories of rock samples during geological time; the long term stress undergone by rocks often causes recrystallisation, the crystals (including the magnetic crystals) becoming aligned in a manner which is related to the stress field present during recrystallisation. Fourth, the under-

standing of rotational remanent magnetisation may help palaeomagnetists to learn how to demagnetise their samples in a more effective way than hitherto. Stephenson's second paper shows that even a stationary specimen in zero steady field, if anisotropic, will acquire an induced remanence during 'demagnetisation'. The practical demagnetisation problem then becomes "How does one tumble a specimen optimally to avoid induced rotational remanence in isotropic samples, gyroremanence in anisotropic samples, and ordinary anhysteretic remanence due to imperfect cancellation of the geomagnetic field?". A problem of some complexity, although it could just have a simple answer.

How trypanosomes change coats

from Mervyn Turner

THE concept of the 'mobile' gene is fast becoming part of the dogma of molecular biology. Precise somatic genetic rearrangements are now being recognised at the basis of the expression of a growing number of genes. From being considered a genetic freak when they were discovered many years ago, translocatable elements in maize have now been joined by immunoglobulin genes and yeast mating type genes in eukaryotes and by the bacterial insertion elements and transposons. The newest additions to the list of mobile genes in eukaryotes are the genes specifying the wide repertoire of surface antigens in trypanosomes. It is now clear that the antigenic variation seen in these parasites of man and his domestic animals is a result of genetic rearrangement (Williams *et al.* *Nature* **282**, 847; 1979; Hoeijmakers *et al.*, this issue of *Nature*, page 78).

Trypanosomiasis affects both man and domestic animals with profound economic consequences across a wide belt of Africa. The salivary trypanosomes are parasitic protozoa normally transmitted cyclically by tsetse flies. The trypanosome survives in the bloodstream of the mammalian host by periodically altering its antigenic profile so that the developing immune response of the host is abortive. The disease is therefore characterised by a relapsing parasitemia with each recrudescence representing the development of immunologically distinct variants. Clones of trypanosomes grown from a single organism can undergo variation, and the same antigens can often be detected in the bloodstream before and after the cyclical transmission, implying that a single organism contains all the necessary information to produce variants, and that variants do not arise by mutation. Although there seems to be no strict

sequence in which the different variants appear, there does seem to be a definable order of priority. The total number of variants which can be expressed by a single clone of trypanosomes is not known but it seems likely to run into hundreds. The variant antigens are glycoproteins of molecular weight about 60,000, which cover the entire surface of the trypanosome. Purified antigens show marked differences in isoelectric point and amino acid composition and the only reported protein sequence data show no homologies within the N-terminal 30 or so residues in four antigens isolated sequentially during the same rabbit infection.

The mechanism by which the information encoding these antigens is stored and expressed is obviously of interest, and two groups have now reported experiments bearing on this point. Williams, Young and Majiwa (*Nature* **282** 847; 1979) partially purified variant-specific mRNA from a clone of *Trypanosoma brucei*, in this case ILPAT 1.2, prepared double-stranded cDNA and inserted this into the plasmid vector-pBR322. A DNA clone containing a 902 base pair coding sequence of variant-specific DNA corresponding to about half the antigen, was identified by hybrid-arrested translation. A probe prepared by nick translation of this sequence was then filter-hybridised to a Southern blot of restriction endonuclease fragments of nuclear DNA from four different clones of *T. brucei* including ILPAT 1.2. Three of these were interrelated, in that they were derived from different relapses within the same strain. The fourth was obtained from an unrelated strain.

In each case the probe hybridised strongly to one or more bands of nuclear DNA, the size of the hybridising fragments differing in each clone of *T. brucei*. The clear implication is that the gene encoding

ILPAT 1.2 is present in all the clones (as expected) but it is flanked by different DNA sequences in each case, implying genetic rearrangement. Moreover, the arrangement is different in all the clones studied not just in the clone expressing ILPAT 1.2.

In this respect the results differ from those of Hoeijmakers *et al.* (this issue, page 78). These workers have prepared cDNA clones from four variants of *T. brucei* in a very similar fashion. The genes corresponding to these variant-specific cDNA clones were then analysed in DNA digests from all four strains by the same Southern blot technique and again the results are consistent with genomic rearrangements. The important and unexplained difference between this and the previous work is that the rearrangement is found only in the homologous variant, that is, the one expressing the variant antigen corresponding to the cDNA probe.

In many ways this fits much better with existing ideas and models for such genetic rearrangements. Hoeijmakers *et al.* interpret their results to show that the same basic gene copy is present in all variants examined, but that the homologous variant seems to show an additional copy of the gene flanked by different DNA sequences and which must presumably be linked with expression of the antigen. The appearance of such an expression-linked copy could be explained by insertion of a transposable promoter next to one of the two copies of the gene which would be found in a diploid organism. Unfortunately information on the ploidy of the trypanosomes is still lacking. A second attractive possible mechanism is gene cassetteing, analogous to the interconversion of yeast mating types. The mating type locus of *S. cerevisiae* can exist in two interconvertible states, α or a , which control the ability of yeast cells to mate and sporulate. The a and α genes are distinct co-dominant entities, which can be regarded as non-homologous blocks of regulatory information. On the basis of genetic evidence it was thought that yeast cells contain a silent (unexpressed) copy of both the a and α information, and that the mating type is controlled by insertion of a copy (or cassette) of one or other of the genes into the mating type locus. Evidence compatible with such a model was presented in *Nature* recently (Hicks, Strathern & Klar *Nature* **282**, 478; 1979; see *News & Views* **283**, 811; 1980) and such a cassette hypothesis could also explain the data presented by Hoeijmakers *et al.* for the variant antigen genes.

If translocation, by whatever mechanism, is involved in antigenic variation, how can the non-random generation of variants be explained? Here the accumulating data on RNA sequences around splicing junctions may provide a clue. Lerner *et al.* (*Nature* **283**, 220; 1980) have tabulated hnRNA sequences at 43 intron/exon splice junctions and although

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the sequences differ it is possible to deduce an idealised 'consensus sequence' using the most commonly found arrangements of bases. By analogy, translocation-specific DNA sequences may exist within the *T. brucei* variant antigen gene repertoire, one such sequence being associated with each gene. The degree of homology with the

ideal 'consensus sequence' could then determine the frequency with which translocation occurred at that particular variant. Clearly sequences from genomic DNA clones will be of immense value in any further studies on the mechanism by which this protozoan Generator of Diversity operates. □

More sensitive immunoassays

from Roger Ekins

A NEW immunological assay method, claimed to be considerably more sensitive than radioimmunoassay, has recently been reported. C. Harris and coworkers have developed an 'ultrasensitive enzymatic immunoassay' (USERIA) applied in this instance to detect cholera toxin and rotavirus (*Proc. natn. Acad. Sci. U.S.A.* 76, 5336; 1979). Radioimmunoassay (RIA) and related methods have played a vital part in endocrinology, and subsequently in other areas of biomedical science since their original development some 20 years ago. Fundamental to these techniques was their exploitation both of the structural specificity characterising antibodies and other specific binding proteins and of the sensitivity of 'signal detection' implicit in the use of radioisotopic labels. These attributes, in combination, provided the basis of assay methods of sufficient sensitivity and specificity to identify and measure the often minute concentrations of many biologically active substances present in biological fluids.

These techniques introduced a new principle into microanalytical methodology: that is, the estimation of the amount or concentration of a substance of interest (the 'analyte') by observation of its distribution between 'reacted' and 'unreacted' moieties following its interaction with a strictly limited amount of the specific reagent (antibody, for example) used. This principle differs from that underlying most traditional analytical methods in biochemistry, in which the estimate of the concentration of an analyte relies on observation of the effect on (or

distribution of) a theoretically unlimited amount of a specific reagent following its interaction with the substance of interest for example, the measurement of a substance by exposure to light and observation of the extent of light conversion to fluorescent radiation. This principle underlies the immunoradiometric (IRMA) — 'radiolabelled antibody' — assay techniques developed a few years later in which excess of the specific reagent (radiolabelled antibody) is allowed to react with the analyte.

IRMA methods — like conventional RIA techniques — rely on a combination of the specificity of molecular recognition characteristic of antibodies (and other specific binding proteins) with the sensitivity inherent in radioisotopic measurement techniques. Nevertheless, although both IRMA and RIA methodologies possess many technical features in common, the contrasting analytical principles which they represent imply that they differ fundamentally both in their sensitivity and specificity characteristics, and in the overall incubation times required to carry out an individual measurement (Ekins in *Radioimmunoassay and Related Procedures in Medicine* 1, 281, IAEA, Vienna, 1978). These distinctions arise largely as a result of the differing influence of the Law of Mass Action on the physicochemical reactions between analyte and specific reagent which constitute the basis of both analytical approaches. In particular, the 'labelled reagent' methods (such as IRMA) offer potential sensitivities many orders of magnitude higher than equivalent 'labelled analyte' assay techniques (such as RIA), primarily because the sensitivity of methods using the latter approach is

essentially limited by the avidity of the specific reagent which reacts with the analyte, as revealed by the equilibrium constant of the reaction. This theoretical constraint does not apply to the sensitivity of 'labelled reagent' methods whose ultimate sensitivity limits are primarily imposed by classical considerations of signal-to-noise ratio, and implicitly by the 'specific activity' (observable events/unit time/unit mass) of the label used.

It is particularly in the context of 'labelled reagent' methodologies that labels other than radioisotopes are likely to offer significantly greater sensitivity and precision, together with absence of bias and shorter assay times. They may also circumvent the problems and hazards of handling radioisotopes which have frequently, though not always legitimately, been advanced as a reason for the ultimate abandonment of the current generation of radioassay methods.

Enzyme-linked immunosorbent assay (ELISA) methods — conceptually identical to IRMA techniques and differing only in their substitution of an enzyme for a radioisotopic label — provide an example of a non-isotopic labelled reagent method which has proved valuable, for example, in agriculture (for the identification of plant viruses) in tropical medicine, and in other biomedical contexts, particularly those in which sophisticated instrumentation is out of place. Nevertheless, conventional ELISA methods have generally — though not invariably — failed to attain the high sensitivities which are implicit in the labelled reagent approach, essentially because the 'noise' (consequent upon 'non-specific' labelled antibody binding) is sufficiently large in relation to the 'signal' (generated by the specifically-bound antibody/enzyme-label complex) to preclude the detection of very low levels of individual analytes.

So the recent description, by Harris and coworkers (*Proc. natn. Acad. Sci. U.S.A. op. cit.*) of their ultrasensitive enzymatic radioimmunoassay is of considerable interest. Aside from the further terminological confusion inflicted on an already chaotic area of nomenclature, their methodological refinement is notable insofar as it utilises a radioactive substrate (^3H -AMP) for the quantitation of the specifically-

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100 years ago

Artificial Diamonds

An unusually large audience gathered at the Royal Society last Thursday to hear Mr. Hannay's account of his artificial diamonds.

The President, after inviting discussion of the paper by Messrs Hannay and Hogarth, observed that probably the large audience

had assembled more especially in consequence of the general interest attaching to the next paper on the artificial formation of the diamond, and he felt that the valuable investigation just detailed showed Mr Hannay to be a person worthy of attention when he claimed to have made even so startling a discovery as that on the face of this next communication. With regard to this the President observed that the attitude of science was always sceptical, and the Society would need ample proof that the metamorphosis of carbon into diamond had been really effected. . .

The President having called for any observations on the notice by Mr Hannay, Mr Maskelyne said that the present differed from the numerous announcements and

other communications that have been heretofore made to scientific societies at various times purporting to record the artificial production of the diamond in this, that here the product so claimed to have been manufactured is really diamond. He had himself proved this by the simple tests of the mineralogist. He had deeply abraded topaz and sapphire with a particle of the substance and abraded them with the greatest ease; the angle of the cleavages of a crystalline fragment sent him by Mr Hannay was the angle between faces of the regular octahedron, and he had burnt a small grain of the substance on a platinum foil with the characteristic glow of the diamond, and without its leaving a residue. From *Nature* 21, 4 March, 421; 1880.

bound enzyme-labelled antibody used in a conventional ELISA method. By this manoeuvre, Harris and his colleagues have succeeded in increasing the signal-to-noise ratio they observe compared with that attained using colorimetrically-based ELISA methods, and have thereby extended the detection limits of this technique to 10^{-16} g (that is 6×10^2 molecules) in the case of cholera toxin. In so doing, they have confirmed the proposition that labelled reagent techniques in general, and labelled antibody methods in particular, are potentially capable of attaining sensitivities many orders of magnitude higher than the 'labelled analyte' or 'saturation assay' methods exemplified by conventional RIA. Aside from the explicit advantages in terms of the reduction in detection limits that this advance offers, a corollary of the far greater sensitivity inherent in 'labelled reagent' methods is the significantly shorter assay incubation times that are required to achieve acceptably precise results.

The technique described by Harris and his colleagues — which might more accurately be described as 'enzyme-amplified' immunoradiometric assay — nevertheless constitutes a somewhat unwieldy approach to the attainment of high signal-to-noise ratios. Nor does it meet the environmental objections to the use of radioisotopes that are increasingly being levelled against both RIA and IRMA techniques. However other forms of label now being developed may offer the same high sensitivities while minimising the complexity of the chemical procedures or of the physical instrumentation required. Two approaches potentially capable of yielding assay sensitivities of the same order as that provided by USERIA rely on the use of chemiluminescent labels as described by Simpson and his colleagues (*Nature* 279, 646; 1979), and of fluorescent markers — particularly those such as the chelated rare earth fluorophores which, as discussed by Soini and Hammilä (*Clin. Chem.* 25/3, 353; 1979) can be readily distinguished from background fluorescence by time resolution techniques. Such alternative labelling methods are under active exploration in several centres. Combined with the exploitation of the *in vitro* hybridoma techniques of antibody production pioneered by Milstein and his colleagues at Cambridge (Milstein & Köhler in *Antibodies in human diagnosis and therapy*, 271, Raven Press, New York, 1977) with which large quantities of monospecific antibodies can be produced, the emergence of simple and reliable assay procedures far surpassing current RIA techniques in sensitivity, precision, speed, specificity, convenience and overall reliability is within sight. The implications of such a prospect in relation to biomedical research, to routine clinical diagnosis, and to related fields such as agriculture, are not difficult to visualise. □

Methane from ridges

from Peter J. Smith

ONE of the more important, but underpublicised, elements of progress in the Earth sciences during the past decade has been the growing appreciation of the crucial role played by hydrothermal fluids in the vicinity of oceanic ridges. Interest in the subject has widened recently, largely because of the physical implications; there can be little doubt, for example, that hydrothermal circulation can go far towards explaining the curious heat flow variations observed across active ridges. Some of the most fundamental consequences of such circulation, on the other hand, are bound to be chemical, for there are few better ways of inducing significant chemical reactions in rocks than to have them bathed in hot chemically-rich fluids.

It is the chemical side which has now attracted Welhan and Craig (*Geophys. Res. Lett.* 6, 829; 1979) — that is to say, the 'chemically-rich' aspect rather than the way the fluids react with the lithosphere. The particular observational site is the crest of the East Pacific rise at 21°N, where recently discovered hydrothermal vents are discharging turbid waters (some black, some white) at temperatures of up to 400 °C. Three samples of these waters were found to be enriched (compared with the ambient seawater) in helium and radon, as are most known hydrothermal fluids whether they come from the Red Sea, the

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Guaymas Basin (Gulf of California) or other sections of the East Pacific rise. But Welhan and Craig find that the 21°N fluids are also highly enriched in hydrogen and methane — and that seems to be an original observation.

As the samples available to Welhan and Craig were contaminated by 'normal' seawater, numerical estimates are likely to be imperfect. Nevertheless, emission from the world oceanic ridge system, estimated by extrapolation from the East Pacific rise data, amounts to 1.3×10^9 m³ of hydrogen and 1.6×10^8 m³ of methane a year. These are, of course, unexpectedly large figures. The mean concentration of deep-ocean methane, for example, is only about 5×10^{-6} ml kg⁻¹, which means that it can all be replaced by new methane from the ridges within a mere 33 years.

Where does the ridge methane come from? Is it, as is the helium, a mantle constituent brought to the crust in the magma rising at oceanic ridges, or is it produced in a reaction between the risen magma and the surrounding seawater? For the time being such questions remain unanswered. In raising them, however, it is curious that Welhan and Craig do not even mention Gold's recent hypothesis to the effect that the outgassing of methane present in the original Earth is still continuing (*J. Petrol. Geol.*, 1, 3; 1979, but given considerable publicity during 1978). Whatever one might think of Gold's hypothesis on the purely intuitive level, it is important that it should be measured against whatever data can be obtained.

Gondwanaland revisited

from Don Tarling

THE Fifth Gondwana Symposium* marked the coming of age of Gondwanaland 16 years after the inception of these symposia. There is now a clear consensus about the final configuration of this supercontinent some 180 million years ago. The unity of South America and Africa as 'western' Gondwanaland and of India, Australia and Antarctica as 'eastern' Gondwanaland has been widely accepted, but the fit of these two pieces has been controversial. New oceanic magnetic anomaly data now provide the required constraints (M.J. de Wit, Witwatersrand; M.W. McElhinny, Canberra; B.J.J. Embleton, CSIRO, Sydney; I. Dalziel, Santa Barbara).

The reconstruction was developed at a workshop in Johannesburg in July 1979 and is broadly similar to that of A.G. Smith & A. Hallam (*Nature* 225, 138; 1970) but

differs in several important respects. 'Eastern' Gondwanaland has been moved much further away from southeastern Africa, leaving room for the Mozambique Ridge, the Agulhas and Falkland Plateaux and so on. Malagasy, although still northerly, is also further from the East African coastline, although possibly not far enough to satisfy me.

The new reconstruction, as do all its predecessors, creates major difficulties in western Antarctica. It is clear that the Antarctic Peninsular existed in the Triassic-Jurassic so it can neither overlap the Falkland Plateau nor run parallel to southern South America (Dalziel). The 'solution' is, for the time being, to break up western Antarctica into discrete blocks that rotated during the Mesozoic evolution of this region. The structural grain of the Ellesworth Mountains is anomalous compared with the rest of Antarctica, but the evidence for its anticlockwise rotation is still largely based on the geometric requirements of the reconstruction itself. However, some palaeomagnetic evidence (K.S. Kellogg, US Geological Survey, Denver) now seems to be consistent with this interpretation. It is intended to use this fit as the base for a geological, geochrono-

*Held in Wellington, New Zealand on 11-16 February, 1980. The Proceedings will be published by Balkema Press at the end of 1980.

logical and resource geology map to be published at 1:10,000,000 in 1981 and de Wit requested data to assist in this. Such a compilation will allow further testing of the reconstruction but it is unlikely that location of the major blocks will be in error by more than a few 100 km. The importance of this map is thus to highlight areas for future research.

A major consideration was the extent of Gondwanaland. Nepal (C.J. Klootwyk, Canberra), Iran (H. Wensink, Utrecht) and Thailand (S. Bunopas & P. Vella, Wellington) were formerly parts of Gondwanaland and there is evidence that much of China (J.B. Waterhouse, Queensland; P. Tasch, Wichita) and possibly some central Asian blocks, with parts of southern Europe (D.H. Tarling, Newcastle upon Tyne), also formed parts of this supercontinent at various times, the degree of floral and faunal interchange between the Gondwanan and Laurasian continents having clearly altered as both continents evolved. The distribution of dinosaurs seems to indicate that Afro-

Indian links persisted well into Late Cretaceous times (E.H. Colbert, Flagstaff) although it is not clear if such links were provided by the Laccadive-Mascarene Ridge or whether access was along the Iranian-Afghanistan coastlines. Palaeobiogeographic provinces formed a significant part of the meeting, with the key glossopterid flora now being divisible into six stratigraphic units that have, so far, been correlated between India and Australia (J.F. Rigby, Geological Survey, Queensland; S.C. Shah, Geological Survey, Calcutta). The presentation of major advances in palynological correlations were predictable, but nonetheless of critical value for the Carbo-Permian (E.M. Truswell, BMR, Canberra) and Permo-Triassic (J. Anderson, Witwatersrand). Although this work solves many problems it also raises others; it destroys the previous, elegantly simple concept of a Gondwanaland drifting through polar regions and hence being glaciated at successive periods in different areas. The age of these ice sheet deposits are almost entirely Sakmarian (Lower Permian) and require much more complex explanations (D.H. Tarling in *Climatic Change*, (Ed. Gribbin) Cambridge University Press, 1978). □

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Vibrations of atoms at surfaces

from John Pendry

THE vibrational properties of atoms and molecules adsorbed on surfaces are determined by the detailed interaction with the surface: whether a molecule dissociates, to what degree an atom is coordinated by surface atoms, whether new species are formed. Knowledge of the way atoms vibrate is an essential complement to our static picture of atomic arrangements. The traditional tool for studying vibrations is infrared absorption, which has yielded much information about vibrations but is limited by the need for the bond to have a strong polar element to be infrared active, and in the case of metal surfaces by the electromagnetic boundary condition which allows only a normal component of the electric field. Electron energy loss spectroscopy (EELS) offers an alternative means of studying vibrational frequencies which to some extent escapes from these restrictions (Ibach *Phys. Rev. Lett.* **24**, 1416; 1970).

Experiments make use of 1-10 eV electrons, which can be monochromated to a few meVs. This represents much worse resolution than infrared can provide and severely restricts the power of the technique. It is unlikely that electron

spectrometry can approach 1 meV resolution and so the technique is mainly concerned with studying strong bonds to relatively light atoms. Despite these considerable restrictions EELS is proving a useful technique which is continually developing both experimentally and theoretically.

There are two ways in which electrons interact with vibrating atoms: through long range and short range forces. If the vibration has an associated dipole moment, an electric field extends far from the surface and operates on an approaching or receding electron for a relatively long period. If a dipole moment is present, this is the dominant mode for inelastic scattering. (Evans & Mills *Phys. Rev.* **B7**, 853; 1973). Because the forces are long range the electron loses energy but remains undeviated in direction. A specular reflection at the surface turns the electron around into an analyser which separates the inelastic component. Until recently this has been the predominant mode of operation of EELS experiments. The requirement of a dipole moment imposes some of the restrictions of infrared spectroscopy and the lack of any substantial momentum transfer to the electron in the inelastic collision limits the information that can be obtained to

vibrations with zero momentum parallel to the surface, as in infrared studies.

The short range interactions are much weaker but their effects can be separated because they can transfer large amounts of momentum to the electron (Ho, Willis & Plummer *Phys. Rev. Lett.* **40**, 1463; 1978). Tuning the spectrometer to the non-specular electrons immediately separates these processes, but at the price of requiring much greater sensitivity. We immediately gain two advantages: the need for a dipole moment is removed; and vibrations with non-zero parallel momentum can be seen. There is a further less obvious gain in information. The matrix element for the scattering involves an incident-wave momentum k_i an exit wave momentum k_f and the displacement of the j th atom, C_j , caused by the vibration:

$$\sum_j \int \exp\{i(\mathbf{k}_i \cdot \mathbf{r} - \mathbf{k}_f \cdot \mathbf{r})\} C_j \cdot \nabla V_j(\mathbf{r} - \mathbf{R}_j) d^3r \quad (1)$$

where V_j is the potential of the j th atom, assumed localised near $\mathbf{R}_j$. If we were investigating the modes of a molecule, it would be useful to know how C_j varied within the molecule. Equation (1) shows that the contribution of each atom to the matrix element has a phase

$$\exp\{i(\mathbf{k}_i - \mathbf{k}_f) \cdot \mathbf{R}_j\}$$

therefore by measuring at different values of $\mathbf{k}_i - \mathbf{k}_f$ it will be possible to identify the individual components of the mode, provided only that we already know the mean positions $\mathbf{R}_j$ of the atoms. That is, we know the phonon 'wavefunctions' as well as the energy levels.

There is an analogy with angle-resolved photoemission experiments in which the energy and momentum of an ejected photoelectron implies by conservation the energy and momentum of the original state of the electron before excitation. (Li, Tong & Mills *Phys. Rev.* in the press). Alternatively knowing both the original and final electron E and $\mathbf{k}$ we can infer the q and ω of the photon. If we read phonon for photon we have the EELS experiment (whether it involves the loss or gain of a phonon). The analogy extends even further: equation (1) gives the matrix element for interaction with a phonon defining atomic displacements C_j . The analogous matrix element for photoemission is

$$\frac{1}{\omega_j} \int \exp\{i(\mathbf{k}_i \cdot \mathbf{r} - \mathbf{k}_f \cdot \mathbf{r})\} \mathbf{A}_j \cdot \nabla V_j(\mathbf{r} - \mathbf{R}_j) d^3r$$

where $\mathbf{A}_j$ is the 'A vector' of the photon field at the j th atom. This close analogy should lead to rapid development of the associated theory of EELS which will be needed to realise the full potential information in the data □

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REVIEW ARTICLE

The role of hormone receptors and GTP-regulatory proteins in membrane transduction

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Cell membrane receptors for hormones and neurotransmitters form oligomeric complexes with GTP-regulatory proteins and inhibit the latter from reacting with GTP. Hormones and neurotransmitters act by releasing the inhibitory constraints imposed by the receptors, thus allowing the GTP-regulatory proteins to interact with and control the activity of enzymes such as adenylate cyclase. This theory may apply generally to membrane signal transduction involving surface receptors.

ADENYLATE CYCLASE, the enzyme that produces cyclic AMP, is part of a complex regulatory system that mediates the actions of hormones and neurotransmitters on their target cells. Structured within the lipid framework of the cell membrane, the enzyme system is composed of at least three classes of components (Fig. 1). Located at the outer membrane surface is the receptor (R) component containing a specific site for binding of hormones and neurotransmitters. At the inner face of the membrane are the catalytic unit (C) and the nucleotide regulatory component (N). The latter contains site(s) for binding GTP and is responsible for mediating the effects of GTP and the various hormones on the activity of C¹. Two types of N units have been distinguished functionally. One mediates stimulation (termed N_s), the other inhibition (N_i) of the adenylate cyclase activity by GTP. As discussed below, each type seems to be linked to separate classes of receptors for hormones and neurotransmitters.

Here I present a theoretical framework for the role of hormone receptors and N units in regulating adenylate cyclase activity. In essence, the theory suggests that the N and R units normally exist separately from C as aggregates or oligomers of an RN complex. In this complex, R inhibits interaction of N with GTP. Hormone binding to R triggers release of the inhibitory constraints imposed on N with resultant enhanced reaction with GTP, followed by breakdown of the oligomers to a monomeric RN complex. The latter reacts with C to form the holoenzyme structure depicted in Fig. 1. Depending on the type of R and N unit attached to C, the holoenzyme exhibits either increased or decreased production of cyclic AMP. In developing this theory, I review evidence for the existence of N_s and N_i and their complexes with R and C, describe the properties of the various components, give recent evidence that RN oligomers exist and discuss the possibility that the theory may be generalised to include R and N units that regulate membrane transduction processes other than adenylate cyclase.

The role of N_s in activation of adenylate cyclase

Before the discovery that GTP is the essential activator of adenylate cyclase² and that hormones enhance the nucleotide's action³, it was thought that hormone receptors interacted directly with the catalytic unit and that fluoride ion, a non-physiological activator, affected the catalytic unit directly⁴. It is now clear that the actions of both hormones (through their

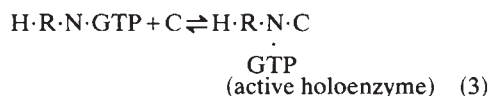
specific receptors) and fluoride ion are mediated through proteins that bind GTP. First identified^{5,6} with GTP-photoaffinity analogues as a heat-stable 42,000 molecular weight protein in detergent extracts of avian erythrocytes, the protein (N_s) has been shown to exist in a variety of cell membranes. Cholera toxin has been particularly valuable for identifying N_s. Long known to stimulate the production of cyclic AMP in animal cells⁷⁻⁹, the toxin potentiates the activating effects of GTP even in the absence of hormones and affects other characteristics of the enzyme that suggest that N_s is its primary site of action¹⁰⁻¹⁴. This was firmly established when it was found that the toxin, which contains in its A₁ subunit an ADP-ribosylating activity, preferentially labels the 42,000-MW protein in the presence of ³²P-NAD (refs 15-17). Cells deficient in N_s by functional criteria also lack the toxin-labelled protein; addition of detergent extracts of membranes containing N_s reconstitutes the ability of hormones, guanine nucleotides, fluoride ion and cholera toxin to stimulate cyclase activity in membranes from cells genetically deficient in N_s but otherwise containing hormone receptors and adenylate cyclase^{18,19}. Thus, judged from several standpoints, N_s is an essential component in the activation of adenylate cyclase. Although its structure remains unknown, N_s must have highly conserved recognition sites that allow it to activate C derived from a variety of cells and to 'couple' with the several types of receptors that mediate the stimulatory actions of hormones.

Table 1 lists a few of the properties of N_s and C when separate and combined (combinations with R are discussed below). C uses MnATP preferentially as substrate^{20,21}. Only when combined with N_s does C use the natural substrate, MgATP, as effectively as MnATP¹². Association of N_s with C is reversible and is driven by the binding of guanine nucleotides to N_s (refs 6, 22).

Properties and role of RN complexes

In classical theories of hormone action, the binding event leads the receptor to adopt a structure that is favourable for action. The relationship between hormone binding (K_D) and action (K_{act}) on adenylate cyclase systems is complicated by the fact that two ligands, hormone and GTP, are required for action². This complexity is exemplified by studies of the glucagon-sensitive cyclase system in liver membranes²³⁻²⁵. Direct binding studies with labelled glucagon revealed that GTP at concentrations required for activation of adenylate cyclase in the

presence of hormone, converted 90% of the receptors to a state with a higher K_D than K_{act} ; the remaining 10% displayed both the kinetic and thermodynamic properties commensurate with hormonal activation of the enzyme. A similar distribution of glucagon receptor states is seen in intact hepatocytes²⁶ which presumably contain sufficient GTP to interact with N_s at the internal face of the cell membrane. A plausible explanation for the effect of GTP on K_{act} and K_D is the following set of reactions (modified from ref. 27)



in which hormone binding and action are initiated at step (1) with resultant formation of an 'activated' state of $N(N^*)$. Reaction of the latter with GTP (step 2) leads to a complex ($H \cdot R \cdot N \cdot GTP$) which preferentially couples with C to form the activated holoenzyme. The negative heterotropic effects of GTP (decreased hormone binding) derive from a lower-affinity form of RN when occupied by GTP and not complexed with C (step 2). Thus, association of the macromolecular components (R, N, C) enhances the binding affinity of the small ligands (hormone and GTP). In this 'uncoupled' equilibrium model, the final concentration of the activated holoenzyme is a function of the relative concentrations of both the macromolecular components and the small ligands. In the overall equilibrium, all RN complexes have equal potential to form complexes with C but the amount of RNC formed is limited by the concentration of C. In the case of the liver system, the latter may be 10% of R and N.

Evidence from β -adrenergic receptor systems supports the above reaction scheme. For example, agonists but not antagonists promote the negative heterotropic effects of GTP on catecholamine binding²⁸⁻³². This is consistent with an ordered reaction in which hormones promote interaction of N in the RN complex with GTP. Evidence that the negative heterotropic effects derive from the RN complex stems from findings that cells lacking C but containing N_s and R display the negative heterotropic effects of GTP on hormone binding²⁰. Furthermore, RN complexes have been isolated following detergent extraction of membranes and separation from C (or NC); such complexes show the effects of GTP on hormone binding seen with intact membranes^{33,34}. Membranes from cells genetically deficient in N_s fail to show not only responses to hormones and guanine nucleotides, but also heterotropic effects of GTP on

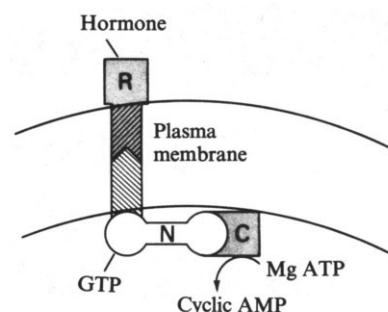


Fig. 1 Schematic representation of the components and organisation of the adenylate cyclase holoenzyme responsible for regulation by hormones and GTP. The receptor (R) is visualised as spanning the plasma membrane and having different segments (indicated by the shaded and cross-hatched areas) which have functions for binding of hormone, attachment to membrane and linkage with the nucleotide regulatory unit (N) that binds GTP. The N unit forms a bridge between R and the catalytic component (C) at the internal face of the membrane.

hormone binding to receptors; addition of extracts containing N_s to such membranes restores the ability of GTP to affect hormone binding³². Further evidence that the N_s unit responsible for activation of cyclase by guanine nucleotides, fluoride and cholera toxin is the same as that linked to R can be deduced from findings that cholera toxin affects the actions of GTP on both hormone binding and cyclase activity¹³. Accordingly, there is no need to invoke different regulatory N units linked to R and C, as has been suggested to explain differing properties of guanine nucleotide effects on hormone binding and adenylate cyclase activity^{27,33-37}. The differences can be explained by heterogeneous forms of N associated with the other components (RN, NC, RNC) having different properties with respect to affinities and actions of guanine nucleotides (Table 1).

The evidence cited above for the existence of RN complexes is based on the effects of GTP on hormone binding. On theoretical grounds, reciprocal effects of hormones on guanine nucleotide binding should also be observed. Recent findings^{38,39} that hormones promote the exchange of bound and free guanine nucleotides at the N_s unit provide further evidence that the R and N units are structurally linked. The functional consequences of this linkage on adenylate cyclase regulation are further discussed below.

It is evident that R units not associated with N units are nonfunctional with respect to adenylate cyclase activation by hormones; free R units in membranes have a lower affinity for agonists than do RN units (when unreacted with GTP)²⁸. Perhaps, free R units are formed from dissociation of RN units and are *en route* to endocytotic removal^{40,41}.

Table 2 lists receptors reported to show negative heterotropic effects of GTP on hormone binding and which are presumed, therefore, to represent RN complexes. Note that they fall into three categories: those involved in stimulation of adenylate cyclase (RN_s), those known to mediate inhibition of the enzyme (RN_i), and those which either do not interact with adenylate cyclase or whose function is unknown (RN_x).

Role of RN_i in the inhibition of adenylate cyclase by neurotransmitter and GTP

In contrast to its adenylate cyclase-activating role, GTP can inhibit some adenylate cyclase systems. First observed⁴² and characterised⁴³⁻⁴⁶ in rat adipocyte membranes, recent studies⁴⁷⁻⁴⁹ indicate that this process differs from the components that mediate stimulation by several lipolytic hormones in the same membranes. For example, adenosine promotes inhibition of adipocyte adenylate cyclase by GTP through processes which differ from the GTP-stimulatory process in their differential susceptibility to effects of sulphhydryl agents, proteases, divalent cations, sodium ions and mercurials.

Table 1 Properties of adenylate cyclase components

Components	Properties	Selected refs
C	Preferential reaction with MnATP as substrate	20, 21
NC	MgATP or MnATP as substrate when activated by Gpp(NH)p*, cholera toxin and NAD in presence of GTP, and by fluoride ion	16, 23
R	Low affinity for hormone agonists; no heterotropic effects of GTP	19
RN or (RN) ^a	GTP reduces affinity of R for hormone agonists. Hormones form tight-binding complex	24, 31, 35
RNC	Same as NC but responds to hormones and Gpp(NH)p; binds GDP tightly in absence of hormones. Hormones stimulate exchange of GDP and GTP at N site	38

*Gpp(NH)p is a GTP analogue that is not hydrolysed to GDP by GTPases in membranes.

The close functional linkage between the adenosine receptor (R-site⁵⁰) and N_i in the adipocyte suggests that there is an RN_i complex for adenosine in fat cells. It appears that RN_i and RN_s complexes in the fat cell interact with a common C unit⁴⁹. Opposing regulation by independent types of RN complexes (Fig. 2) may be widespread, particularly in cells that have adenylate cyclase systems governed by the endocrine and neuroendocrine systems. Receptors for such neurotransmitters as opiates, dopamine, catecholamines (α -adrenergic) and cholinergic agents (muscarinic) also mediate inhibition of adenylate cyclase by a GTP-dependent process⁵¹⁻⁵⁴. In common with the adenosine receptor linked to N_i in adipocytes, these receptors are sensitive to sodium ions. Interestingly, in the few cases examined, negative heterotropic effects of GTP on agonist binding are observed with the same membranes showing the GTP dependency of agonist inhibition of adenylate cyclase; sodium effects on agonist binding are also observed (Table 2). These findings suggest, by analogy with the role of RN_s complexes in the stimulation of adenylate cyclase, that RN_i complexes are involved in the inhibitory process.

The structure of RN

The technique of target size analysis has recently been used to examine the size of adenylate cyclase systems in various conditions in their membrane-bound form (for application and theory see ref. 55). Results obtained from liver⁵⁶, adipocytes and turkey erythrocyte membranes are shown in Table 3. The minimal size unit expressing activity is that obtained with MnATP as substrate and no activating ligands; it is presumed to represent C. The size increases significantly as N_sC is produced with pre-activation by guanine nucleotides or fluoride ion. Activation by hormones and GTP which should produce the holoenzyme (RNC), is accompanied by a further increment in size commensurate with this premise. In the case of the liver system, the sizes of the regulatory complexes (RN_s) were estimated from studies of the binding of labelled glucagon in the presence of GTP and from the 'ground-state' cyclase activity, that is the size of components present before coupling. Both studies indicate that the RN_s structure in the liver has a MW in the range $6-13 \times 10^5$, which is three to six times the estimated size of the RN unit associated with the holoenzyme. The conclusion from these findings is that RN_s exists as oligomers when not linked to the C unit. Note in Table 3 that the unit (presumably RN_i) responsible for inhibition of adipocyte adenylate cyclase by adenosine and GTP is significantly larger than the oligomeric RN_s unit that mediates the stimulatory effects of hormones and GTP on the same enzyme system. Although target analysis

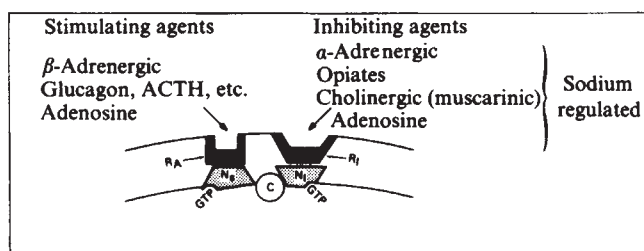


Fig. 2 Schematic representation of dual regulation of adenylate cyclase systems by stimulatory and inhibitory hormones and neurotransmitters. Depicted in the model are two classes of receptor (R) one (R_s) mediating hormone effects through stimulatory nucleotide regulatory units (N_s) and another (R_i) mediating inhibitory effects through linkage with an N_i unit that binds GTP and inhibits adenylate cyclase activity. The R_iN_i units require Na^+ for the effects of the various inhibitory agents on adenylate cyclase (C) activity.

cannot give the structure and composition of the target, it can be inferred from the size differences that the stimulatory and inhibitory processes reflect different structures that are multiples of the RN units comprising the holoenzyme.

Although the sizes of the adenylate cyclase system in turkey erythrocytes display increments with increasing regulatory complexity, this system does not show the oligomeric structures of RN_s ; the functional size remains identical before and after activation with catecholamines and guanine nucleotides. Possibly, R, N and C are already assembled in the holoenzyme structure due to prior activation of the enzyme system during isolation of the membranes. Pre-assembly may explain some notable differences in kinetic behaviour of the turkey and liver adenylate cyclase systems (discussed below). A pre-assembled unit is also consistent with the report⁵⁷ of a linear relationship (rather than the usual hyperbolic relationship) between hormonal activation and receptor occupation in the turkey system.

A model for hormone and GTP action on adenylate cyclase

The existence of oligomeric complexes of RN provides a structural basis for the uncoupled equilibrium reactions described above. For illustrative purposes, the model depicted in Fig. 3 shows a tetrameric structure of RN units existing in two

Table 2 Hormone receptors regulated by GTP (the RN complex)

Receptor type	Source	Type of N unit*	Comments	Selected refs
Glucagon	Rat liver	N_s	GTP = GDP; Gpp(NH)p and Gpp(CH) ₂ p less potent	24
Catecholamines (β -receptors)	Several cell types	N_s	Divalent cations promote binding of agonists; only agonist binding affected	28-32
Prostaglandin E	Thyroid, frog erythrocyte	N_s	Binding promoted by Ca^{2+} ions	98, 99
Dopamine	Corpus striatum	N_s, N_x	GTP = GDP > Gpp(NH)p; agonist specific	100, 101
Muscarinic	Canine and rat myocardium	N_i	Methacholine inhibits GTP effects on β -receptor; Na^+ probably affects binding	102, 103
	Neuroblastoma x glioma cells	N_i	Mg^{2+} enhances agonist binding	105
Catecholamines (α -receptors)	Brain	N_x	GTP = Gpp(NH)p > GDP; Na^+ affects agonist binding	106, 107
	Rat liver	N_x	Agonist specific	83
Angiotensin	Adrenal cortex	N_x	Agonist binding affected by Na^+ in same manner as by GTP	84
Opiates	Brain	N_x	Na^+ affects binding of agonists and antagonists. Two distinct opiate receptors	52, 104, 108

In all cases, addition of guanine nucleotides decreases binding of hormone or neurotransmitter to specific receptors in isolated membrane preparations.

* N_s is N unit linked to stimulation of adenylate cyclase; N_i affects inhibition of the enzyme; N_x is an N unit that is either not related to cyclase activity or has an undetermined relationship to a specific signal-processing system in the cell membrane.

configurations: an unoccupied structure (A) which favours binding of hormones but not GTP, and a hormone-induced or stabilised structure (B) which favours reaction of GTP with the N component (equivalent to N^* in the previous reaction scheme). Reaction with GTP results in dispersion of the oligomer to monomers (at this stage the negative heterotropic effects of GTP occur) which uniquely react with C to form the holoenzyme that converts MgATP to cyclic AMP. In broad terms, this 'disaggregation-coupling' model for hormone and GTP action can be likened to the manner by which cyclic AMP controls through its receptor the activity of protein kinase; the latter involves a change in the association of dimeric regulatory and catalytic subunits⁵⁸. In the case of hormone receptors associated with adenylate cyclase systems, their interaction with N units in the oligomeric state constrains the ability of N to react with GTP, thus preventing the formation of the 'active' form of the regulatory N unit (N_s or N_i). It follows that cyclase systems devoid of receptors linked to N should display high reactivity with GTP. This has been reported⁵⁹ recently with a strain of HeLa cells deficient in β -adrenergic receptors; when the same cells become enriched with receptors, catecholamines are required to restore the level of activity seen with GTP alone in receptor-deficient cells.

The disaggregation-coupling model has several other important features that serve to distinguish it from previous theories of hormone action⁶⁰⁻⁶². First, assuming that the oligomers of RN are unreactive with C, it provides a means for functional compartmentalisation of RN units from C and explains the observed dependency of the reaction on both hormone and GTP. Second, an oligomeric structure of RN allows for homotropic subunit interactions such that minimal occupation by hormones may cause near maximal production of the functional activating 'signal' (the RN-GTP monomer) in the presence of saturating concentrations of GTP; marked activation of adenylate cyclase with minimal occupation of receptors has been observed with several cyclase systems^{24,57,63,64}. Thus, although full occupation of receptor subunits may drive complete coupling of RN with C, coupling may occur even when these units are not occupied with hormone. Third, because the oligomeric RN structure is inert with respect to activation of adenylate cyclase, aggregation of uncoupled regulatory units is a means of 'turning off' the reaction cycle. How 'uncoupling-aggregation' is accomplished and what factors control this part of the cycle remain unknown. Possible candidates are cytosolic factors reported to affect adenylate cyclase activity⁶⁵⁻⁶⁹. Additionally, factors intrinsic to or associated with the plasma membrane may be essential in the aggregation-disaggregation cycle; these factors could include cytoskeletal elements⁷⁰ which may stabilise the aggregated units in the membrane; interestingly, agents that disrupt cytoskeletal structures have been reported^{71,72} to enhance cellular production of cyclic AMP in response to hormones.

Emphasis has been placed recently on regulation of hydrolysis of GTP by a GTPase putatively associated with the N_s unit^{14,73,74}

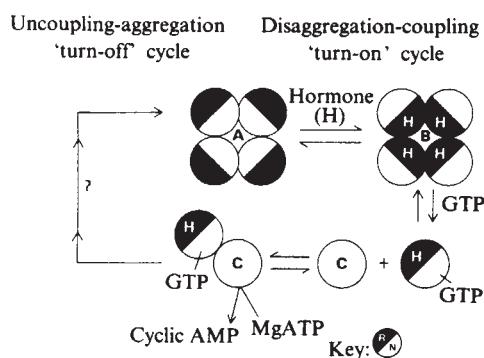


Fig. 3 A model for coupling of the receptor-nucleotide regulatory units (RN) to the catalytic unit (C) of adenylate cyclase and the role of hormone and GTP in this process. See text for detailed description.

Table 3 Functional sizes of adenylate cyclase components

Enzyme source	Components (MW $\times 10^5$)				
	RN_s	C	N_sC	RN_sC	R_iN_i
Rat liver: R = glucagon	6-13*	1.5	2.3	3.5	—
Fat cell (rat) R = catecholamine, ACTH R_i = adenosine	13	†	2.3	†	>13
Turkey erythrocyte R = catecholamine	ND	0.9	1.8	2.5	—

Sizes were determined by target size analysis using high energy irradiation. See ref. 56 for methods for determining size and rationale for assigning components to size. Data for fat cell and turkey erythrocyte data are unpublished. ND, Not detected.

* Estimated both from GTP-sensitive glucagon binding and adenylate cyclase activity of 'ground-state' enzyme.

† Not determined.

and the role of this regulation in turning-off the GTP-activation process. In turkey erythrocyte membranes, catecholamines and cholera toxin affect GTPase activity and GDP is a potent inhibitor that binds tightly to N_s (refs 38, 39). The differences in the kinetic characteristics of the liver cyclase system in its response to GTP and Gpp(NH)p are consistent with a GTPase being involved in its dynamic characteristics⁷⁵. However, GDP has been shown to stimulate liver cyclase activity in the presence of glucagon^{1,36,76}; stimulatory effects of GDP have been reported also in the presence of ACTH on the adipocyte adenylate cyclase system⁷⁷. In view of these findings it is not clear that a GTPase turn-off mechanism^{78,79} (which necessarily requires that GDP binds tightly and acts only as inhibitor) can adequately explain the dynamic properties of all adenylate cyclase systems. Perhaps the difference in the properties of the turkey, liver and adipocyte systems is related to the lack of an oligomeric structure of RN in isolated turkey erythrocyte membranes.

The topographical relationship between the RN oligomers and C in the plasma membrane and the role of membrane lipids in this relationship remain unknown. Studies with phospholipases have shown that hormone action on adenylate cyclase remains intact even after 85% of the membrane phospholipids have been digested; substantial loss in action occurs only when a fraction of the remaining phospholipids is hydrolysed⁸⁰⁻⁸². These findings could mean that RN oligomers and C are in close proximity and possibly in selective domains of interacting phospholipids; the bulk of the phospholipids do not seem to be involved in assembly of the holoenzyme.

Generalisations and problems

If the notion is accepted that the effects of GTP on hormone binding to receptors are due to disaggregation of oligomers of receptors linked to N units, then the theory cited above may apply generally to all hormone-regulated systems that illustrate these effects, even those N units (N_x) not linked to adenylate cyclase. The latter include such GTP-affected receptors as angiotensin receptors in the adrenal medulla⁸⁴ and α -adrenergic receptors in liver⁸³ (see Table 2). Recent findings⁸⁵ that insulin activates a cyclic AMP-independent protein kinase in sarcolemma membranes by a process regulated by GTP is an interesting example of a potential N_x -mediated process unrelated to adenylate cyclase.

The possibility that different N units mediate the actions of hormones raises interesting new questions. Can the properties of the same receptor be modified by interaction with different types of N units? What determines the interactions of receptors with a particular type of N unit? The pertinence of these questions is exemplified by reports that catecholamines, acting through α -adrenergic receptors (by pharmacological criteria), both stimulate⁸⁶ and inhibit^{51,54} cyclic AMP production, and induce effects unrelated to adenylate cyclase activity⁸³. Other hormones exerting multiple effects on membrane processes

include adenosine⁴⁸⁻⁵⁰, dopamine^{53,87-89}, opiates⁹⁰⁻⁹², vasopressin^{93,94} and serotonin⁹⁵. Pharmacological studies (specificity, potency, antagonist or hormone binding to receptors) alone may not identify the type of receptor mediating these processes. Heterotropic effects of GTP on agonist binding indicate linkage of receptors to an N unit but do not identify the type of N unit. I have noted in Table 2 that certain RN complexes are affected by sodium ions, others by divalent cations, and that guanine nucleotides have different potencies on agonist binding. Perhaps such differences can be used to classify the N unit associated with the receptor. Clearly, what is necessary in future research in this area is to develop assay methods that identify unequivocally each type of N unit. Testing of biological effects is not necessarily the means of determining the type of RN unit. A bizarre example of problems encountered is the action of cholecystokinin on pancreatic acinar cells⁹⁶. In the cell the hormone stimulates zymogen secretion, calcium release and cyclic GMP production, but not the production of cyclic AMP; after breakage of the cell, the hormone stimulates adenylate cyclase in a GTP-dependent fashion.

Although other explanations are possible, these findings raise the possibility that 'redistribution' of receptors and N units occurs on cell breakage owing to the breakdown of stationary domains that normally segregate these units in the cell. 'Lateral domain redistribution' might be a physiologically regulated process due either to changes in the structural relationship of the cytoskeleton to the ordered domains or to propagated disturbances in the membrane structure. A possible example of the

latter is the report⁹⁷ that catecholamines, acting through a β -adrenergic receptor, stimulate a phospholipid methylating enzyme by a process which is affected by GTP but which does not involve the production of cyclic AMP; associated with methylation is an apparent 'flip' of the internally methylated lipids to the outer face of the membrane and a change in lipid microviscosity. Pleiotropic effects of the hormone could thus be generated by localised changes in lipid structure being propagated laterally and modifying the postulated domain structures of the membrane. In any event, these findings question the commonly held view that β -adrenergic receptors are singularly linked to stimulation of adenylate cyclase. Moreover, the dependency of the hormone effect on GTP suggests again the versatility of the N units in mediating the actions of hormones.

In conclusion, the classical notion of receptor alone controlling the events related to hormone and neurotransmitter action on adenylate cyclase is no longer valid. The constraining role of hormone receptors postulated here differs from the role of the receptor postulated in other theories of hormone action. More importantly, the theory places in perspective the role of another set of regulatory proteins (the N units) hitherto given relatively scant attention, particularly with regard to their apparent multiple and fundamental roles in the regulation of membrane-associated processes. I hope this rather brief article will stimulate investigation of the new problems to be faced in ascertaining both the structures of the GTP-regulatory proteins and how they function in the transduction of hormone binding at cell membrane receptors into physiological action.

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ARTICLES

Cometary collisions on the Moon and Mercury

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Unusual swirl patterns of bright and dark material on the Moon and Mercury are proposed to be remnants of collisions with gas/dust-rich regions within a cometary coma. This interpretation provides important new clues for understanding cometary fine structure, impact effects of low-density material, and the origin of certain pronounced magnetic anomalies.

ALTHOUGH cometary impacts have long been assumed for inner Solar System bodies such as the Moon and Mercury^{1–3}, there has been little evidence for distinguishing such an event from a meteoroid impact. We propose that the enigmatic bright and dark swirls which cross portions of the lunar farside^{4,5} may be best explained by an impacting comet complex. The strong magnetisation of at least one swirl (Reiner γ) (ref. 6) and the close association of other swirls with lunar regions containing strong magnetic anomalies⁷ suggest that these features were magnetised in the impact. We argue that the swirls are young deposits, implying a recent cometary impact ($< 10^8$ yr ago) and ruling out an active lunar dynamo^{8–10} as the source of their magnetising field. Rather, this field may have been of cometary origin, perhaps amplified during the impact^{11,12}.

Bright/dark swirl patterns occur in three regions of the Moon. One of the best known examples is Reiner γ , which is near the western limb of the nearside (5° S, 60° W). The concentrations of patterns near Mare Marginis (15° N, 90° E) and Mare Ingenii (35° S, 180° E) are more impressive. Bright/dark swirls have not previously been studied in detail, but have generated several possible interpretations including nué ardente deposits¹³, antipodal effects of major basins⁴, volcanically derived sublimates⁴, secondary impact effects⁶, unusual secondary cratering phenomena associated with a cometary impact⁵, and selective preservation of albedo (crater rays) controlled by local enhancement of magnetic fields⁷. More detailed examination, however, reveals features that are suggestive of remnants of the impacting nuclear region of a comet.

Swirl patterns range in size from nearly 10 km to < 50 m across and form a variety of characteristic geometries: ribbon-like patterns, open loops and closed loops. As Fig. 1 shows, these patterns are commonly crossed by dark lanes. Both dark and bright patterns drape relief such as crater walls and rims and cross both the highlands and mare terrains (Fig. 2). At the highest resolutions available (~ 10 m), alteration of the surface (scouring) is not visible. Rather, the patterns represent diffuse brightening/darkening of unmodified terrains and commonly exhibit sharply defined boundaries over 50–100 m scales. In several examples, higher albedo regions correspond to sloped surfaces (for example, walls of degraded craters), whereas lower

albedo regions correspond to low-lying regions (for example, crater floor). Under high illumination, such patterns form a distinctive bright ring that strongly contrasts with other adjacent degraded craters. Ring patterns occur, however, in plains regions, not in association with changes in relief. Consequently, there may be several processes contributing to the formation of swirls.

Similar patterns are recognised on Mercury near latitude 20° N, 47° W and 4° N, 35° W. Because different phase angles and high-resolution images of these regions are unavailable, it is impossible to verify this identification in detail. Nevertheless, the bright loops and swirls noted at these locations are very similar to the gross patterns found on the Moon.

Although detailed photometry of the farside swirls is not available, several observations can be cited. Bright patterns occur on both dark (mare) and light (highland) surfaces. Apollo and Lunar Orbiter photographs under different illuminations and phase angles reveal that the bright patterns are strong forward reflectors (large phase angles) in contrast with most crater rays. This property, along with their characteristic pattern, make swirls easily identifiable. Dark lanes do not share this property, and under large phase angles, typically exhibit a reflectivity comparable to the surrounding terrains. Microdensitometer tracings reveal that the dark lanes are not simply an effect of contrast with the bright swirls, but exhibit a lower reflectivity under full illumination.

Longer wavelength measurements are available only for the nearside pattern, Reiner γ . Earth-based 70 cm (ref. 14) and 3.8 cm (ref. 15) radar data reveal no significant enhancements associated with Reiner γ . IR data during lunar eclipse¹⁶ reveal a possible 'cool region' that includes this pattern. Available data from the Apollo 17 Infrared Scanning Radiometer (ISR) provide much higher spatial resolutions (W. Mendell, personal communication) and also indicate no thermal enhancements. Consequently, Reiner γ exhibits a near-surface population of blocks resembling the average regolith. These data raise serious problems in the creation of a magnetic anomaly at this site, regardless of the proposed field source and remanence mechanism.

Swirl patterns are concentrated within two fan-shaped regions that open to the east (Fig. 3). Patterns near Mare Marginis converge near the 11-km diameter crater Goddard A, whereas patterns farther east converge near Mare Ingenii, perhaps

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towards a fresh 8-km crater on the wall of O'Day. The Reiner γ patterns may represent an eastern extension of the latter system.

The areal coverage, fine structure and shape of swirls change with distance from the western convergent zone. Near Mare Marginis, the greatest areal coverage by swirls occurs within ~ 300 km of Goddard A. Individual patterns typically cover 400 km^2 . Beyond 300 km east of Goddard A, swirls exhibit finer patterns each typically covering $< 100 \text{ km}^2$. In contrast, swirls abruptly disappear beyond 250 km west of Goddard A. Figure 3 also reveals that characteristic pattern shape changes in relation to Goddard A. Swirls are broad, arcuate loops roughly concentric with Goddard A to the west, but form parallel patterns within 400 km east of Goddard A. At greater distances, individual patterns form tightly closed loops, rings and narrow ribbons.

Although individual swirl patterns cross highland relief, there seems to be some topographic control at both fine and broad scales beyond 400 km from Goddard A. At fine scales (< 20 km), bright rings encircle a dark interior and commonly coincide with medium-size craters (~ 10 – 20 km diameter) having smoothly sloping walls. At broader scales, swirl patterns cluster within large craters and basins such as Fleming, Ingenii, Van de Graaff and Aitken.

We argue that swirl patterns are very young features. They clearly overlay the well preserved farside craters King and Necho, and appear to cross rays from Giordano Bruno. Crater statistics for King crater¹⁷ reveal a production population down to craters at least 20 m in diameter, thereby indicating a very young age for the swirl patterns ($< 10^8$ yr). The stratigraphic relation with Goddard A, however, is more complex. West of this crater, rays appear to roughen or "feather" the bright swirl patches and cross dark lanes. Nevertheless, swirl patterns clearly superimpose ejecta deposits elsewhere. Such relations suggest swirl formation contemporary with Goddard A.

Recency of swirl formation is also indicated by the general absence of small, subsequent craters that excavate underlying contrasting material. Important exceptions west of King Crater exhibit a highly unusual morphology: bright-rayed and dark-rayed subdued dimple craters. Similarly, clusters of small, rayed craters superpose sections of the ejecta facies of Goddard A. These exceptions may be related to impacting material associated with swirl formation.

The crossing of relief, the absence of visible surface scouring and the photometric/IR properties suggest that the swirl patterns represent either a deposit or a physical alteration of the uppermost regolith layers. The distribution and geometry of patterns suggest a relation with the formation of the crater Goddard A and an exogenic origin that is unrelated to secondary cratering. These observational constraints are consistent, however, with highly dispersed material impacting the surface before and soon after Goddard A. Such a sequence is expected for a cometary impact where the tenuous inner coma should both precede and follow impact by the nucleus by 50–100 s. More specifically, the swirls may represent remnants of collisions by gas/dust-rich zones within the inner coma. These zones may be fine-scale counterparts to the streamers and knots commonly observed in the inner nuclear regions of comets as a result of sporadic explosions of material (ices, solids and gases) from the nucleus¹⁸.

The detailed structures of the inner coma and inner nucleus regions are, however, poorly understood. Number densities of the primary parent molecules of a cometary atmosphere¹⁹ suggest that the total coma mass (gas plus dust) within 200 km of the nucleus could range between 10^9 and 10^{10} g (This value assumes an isotropic coma where the number density varies as the inverse square of the distance from the nucleus. The parent components are assumed to approximate values for Comet K where $\text{H}_2\text{O}:\text{C}_2:\text{CN}:\text{CH}_3\text{CN}:\text{HCN}$ relative abundances are 17:6:2:0.5:1.0(?):2(?), respectively. The absolute number density of H_2O is taken as 2×10^{11} for a production rate of 3×10^{29} molecules s^{-1} and a lifetime of 2×10^4 s. It is further assumed that the gas/dust ratio is unity.). If the swirls cor-

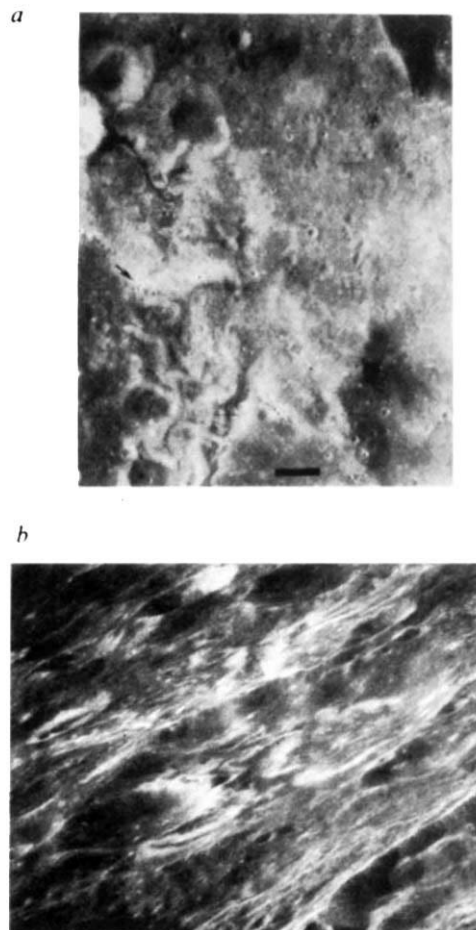


Fig. 1 *a*, Swirls 130 km west of King Crater at longitude 125°E , latitude 5°N showing characteristic looping pattern. Annulus of bright material at top coincides with a subdued crater. Arrow identifies ejecta from Necho crater that are crossed by dark lanes of the swirl patterns. Scale bar, 5 km. *b*, Swirl patterns overlapping rim of 23 km-diameter crater at longitude 110°E , latitude 11°N . Preservation on sloped surfaces suggests recent formation.

respond to gas/dust-rich zones, then this mass is not distributed isotropically, but is locally concentrated. Within 200 km of Goddard A, the total area covered by the swirls is $\sim 3,600 \text{ km}^2$, which represents nearly 10^5 km^3 . Thus, average mass densities in these zones may have ranged between 10^{-10} and $10^{-11} \text{ g cm}^{-3}$. Higher densities (10^{-8} – $10^{-9} \text{ g cm}^{-3}$) could be expected near the centre of major zones. Such values correspond to terrestrial atmospheric densities between altitudes of 90 and 120 km.

Although very tenuous, the derived gas densities are sufficient to heat, ablate and incinerate small meteors during entry into the Earth's atmosphere at velocities comparable to those expected, even for a low-speed cometary impact on the Moon ($\sim 20 \text{ km s}^{-1}$ ref. 4). This suggests that thermal alteration of the regolith may have occurred in the swirl areas and that the dynamic pressures (~ 0.1 bar) may have been sufficient to transport material on a micro-scale. Topographic control of reflected shock waves could locally enhance this process. In addition, if cometary dust particles are electrically charged and become confined within gas jets from the nucleus, their impact on the regolith at hypervelocities would generate an anomalous population of glasses and micrometre-sized iron particles. Both processes can alter the reflectance properties of the surface, which are sensitive to the mineralogy and petrology of the uppermost regolith²⁰. Consequently, the bright/dark swirl patterns are thought to represent high-velocity imprinting of the coma fine-structure by very fine-scale erosion/deposition and by the build-up of glassy materials through ablation of regolithic fine structure and impacts by cometary dust.

The recent discovery by Hood *et al.*⁶ that the nearside swirl feature Reiner γ coincides with a strong magnetic anomaly, and the suggestion that the swirls are generally in regions of strong remanent magnetic fields⁷ indicate that at least some of the lunar magnetic anomalies are of external origin, and are perhaps generated by cometary impact. Recent formation of these features precludes an origin by solar-wind stand off as proposed by Hood *et al.*⁶. Discussions of lunar anomalies must always treat two distinct problems: origin and strength of the magnetising field, and the nature of the remanence mechanism in the lunar rock which allowed a permanent magnetisation to be acquired^{21,22}. If remnant fields are associated with the swirls, only two courses are plausible for such a correlation: either the remanent fields predated the deposition, and merely served to guide the incoming coma material into a nonuniform pattern on the surface; or the field was present only during the emplacement of the deposits, during which time some remanence mechanism operated to generate the anomalies coincident with the swirls. In this case the magnetic fields are associated with the comet, or are generated during the impact¹².

The first hypothesis is untenable. To channel the plasma flow effectively, the remanent magnetic field B must exceed the value $B > (2\mu\rho)^{1/2}V$ at least one ion-gyro radius above the surface, where ρ and V are the mass density and flow velocity of the partially ionised coma gases, and μ_0 is the permeability of free space. Taking $V = 20 \text{ km s}^{-1}$ and $\rho = 10^{-10} \text{ g cm}^{-3}$ gives $B > 10^{-5} \text{ T} = 0.1 \text{ G}$. Since ρ may have been as large as $10^{-8} \text{ g cm}^{-3}$ in coma jets, lunar remanent fields of 0.1–1 G would have had to exist on large scales over the lunar surface at the time of the impact to channel the flow. Compression of the local field would ease this requirement somewhat, but not by more than an order of magnitude. As no evidence exists for broad-scale remanent field strengths of this magnitude on the moon, we reject this guided-plasma model.

Thus we argue that the remanent field now seen in swirls, such as Reiner γ , was acquired in the surface at the time of a comet

impact. It is likely that the magnetising field was intrinsic to the comet, perhaps generated in the nuclear region by a dynamo-like process²³ when the comet is close to the Sun ($< 1.5 \text{ AU}$) and thus very active, or simply due to the unipolar induction interaction of the coma with the solar magnetic field^{24–26}. In any case, the cometary field will probably be amplified as the coma is compressed against the lunar surface¹¹. However, the degree of amplification is not arbitrarily large, as current-driven plasma turbulence will create anomalous resistivity in the compressed region as the current density rises, and the electric fields will saturate. Although no data are available on field strengths in cometary comas, the poloidal field conversion model²³ gives values of 10^{-2} G , and allowing for the compression effects discussed by Gold and Soter¹¹, field strengths of 1–10 G should be present at the lunar surface during the impact for a considerable period. If the coma is $\sim 10^4 \text{ km}$ in size and hits the Moon at 20 km s^{-1} , large fields could be expected for times of the order of 10^3 s or more as the incoming tail continues to feed plasma and kinetic energy to the current systems flowing parallel to the lunar surface long after the nucleus has impacted. Apparently strong, relatively long-lived magnetic fields can be expected to be present at the lunar surface during such an event.

The critical problem in any impact-related generation of magnetic anomalies is the identification of a suitable remanence acquisition process. Shock remanence²⁷ is known to be effective in lunar surface materials when the shock pressures are in the range of 5–20 kbar. Such pressures are not likely to accompany swirl formation unless generated by individual microparticle impacts accompanying colliding gas/dust-rich regions. Moreover, these pressures could not be propagated beyond the rim region of the impact by the nucleus. Thermal remanence may be acquired if the jet of coma gas and dust impinging on the surface heats and alters the regolith in the presence of the ambient field. In this case each particle must cool through its Curie point while the field is still present. Such a process can occur if the impacting dust particles locally heat small volumes of

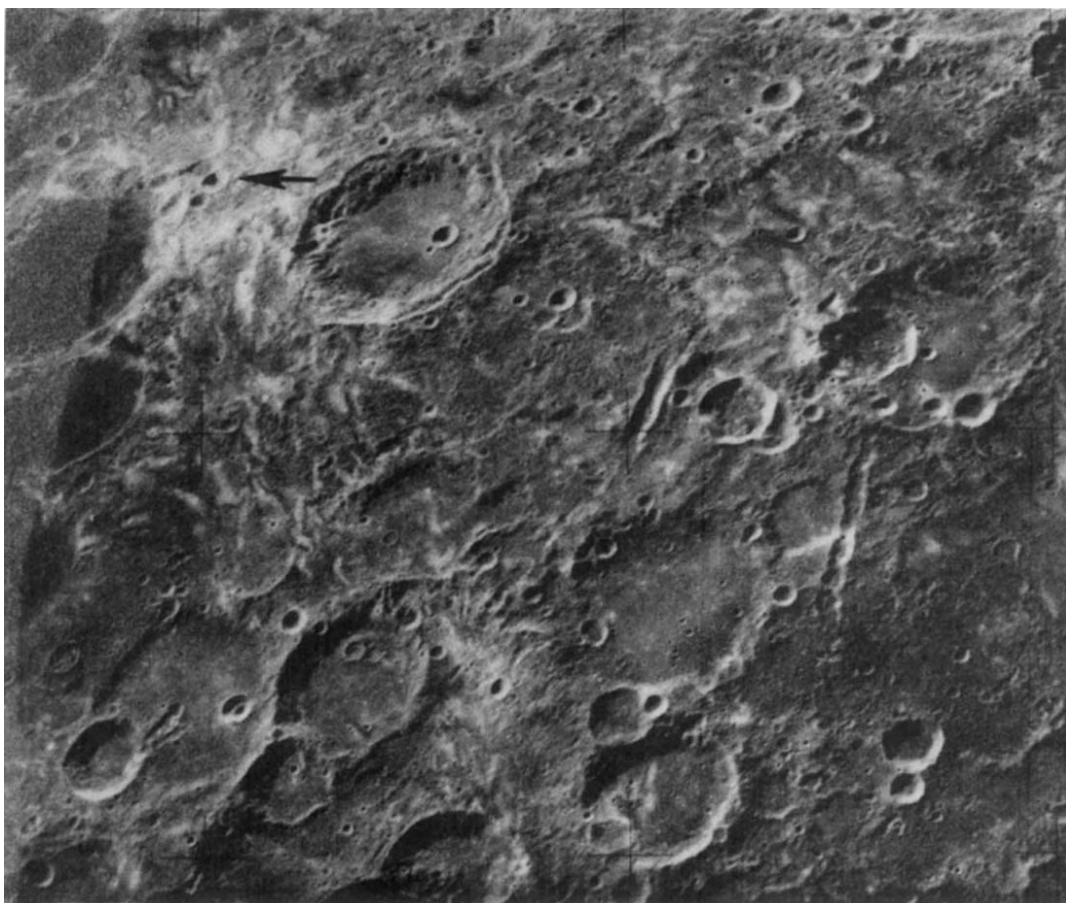


Fig. 2 Overview of region near Mare Marginis. Arrow identifies the crater Goddard A proposed to represent impact remnant of nucleus.

Fig. 3 *a*, Distribution of swirl patterns near Mare Marginis and farther east showing general concentration in a fan-shaped zone open to the east. Subarcuate patterns west of Goddard A (●) may represent hydrodynamic contact surface. Swirl patterns are proposed to be the result of fine-scale surface alteration by colliding gas/dust-rich regions within the inner coma. *b*, Distribution of swirl patterns near Mare Ingenii illustrating similar fan-shaped concentration. Reiner γ may be an eastern extension of this system.



soil, producing free iron droplets on a micrometre scale that cool in a fraction of a second and hence, acquire thermoremanence easily¹². However, it is difficult to explain specific features of the Reiner γ anomaly in this way. For example, the magnetisation direction for that swirl has a large component normal to the lunar surface⁶, whereas arguments based on a frozen-field MHD model suggests that the amplified cometary-field would be primarily tangent to the surface¹¹. However, the coma jet may have its own current system and magnetic field²³, in which case vertical fields may occur. A principal uncertainty is the inferred present surface strength of the remanent magnetic field at Reiner γ .

Using the data of Hood *et al.*⁶ and evidence that the swirl deposits are very thin (<1 m), we find a required regolith magnetisation I of $I > 0.8$ e.m.u. g^{-1} for a density of 2 g cm^{-3} , which exceeds by four orders of magnitude the stable component of remanence in lunar rocks, and by two orders the highest value of remanence observed in any lunar sample (G. W. Pearce, personal communication). This places severe constraints

on the composition of the regolith in the swirls. If we assume that the sampled lunar rocks constrain the remanence value in Reiner γ , then depths of alteration of 1 km or more are required to produce the Reiner γ magnetic anomaly. As the regolith depth of mare surfaces is <6 m, the remanence at Reiner γ must be much higher than any of the values yet found in the lunar samples. This demands either a very high free iron content, very high (perhaps saturation?) remanence in the iron grains, or both. The impacting coma gases (H_2O , CO_2 , and OH^-) also might alter the chemistry and mineralogy of the swirl areas, perhaps producing local concentrations of magnetite in the dark areas of the swirls.

If these interpretations are correct, then several statements can be made about the proposed comet. From scaling relations between crater diameter and impact energy²⁸, the size of Goddard A suggests a nucleus 200–500 m in diameter. Note that the resulting crater is not anomalous in appearance. The subarcuate patterns disappearing beyond 200 km west of Goddard A may correspond to a hydrodynamic contact surface,

which develops by collision of the sub-solar cometary atmosphere and the solar wind¹⁸. Broad swirls within 100 km of Goddard A correspond to the inner nuclear region of gases and dust, whereas the elongate and parallel patterns east of Goddard A delineate matter in the process of being swept into the flow patterns of the tail. Beyond 200 km from Goddard A, the remaining patterns indicate concentrations of gases controlled by magnetic field lines within the comet^{29,30} and/or by gases sublimating from ice bodies dislodged from the nucleus. The latter process could account for the unusual dimple-shaped bright and dark-rayed craters associated with certain deposits west of King crater. The general restriction of the swirl patterns within a fan-shaped zone reflects the projected form of the streamlined inner coma on the lunar globe. Clusters of small primary craters superposing the ejecta facies Goddard A and a few swirls suggest that a myriad of centimetre/metre-size meteoroids may have accompanied the event.

We take the correlation of a strong magnetic anomaly with one of the swirls, Reiner γ , as evidence for a strong magnetic field within the comet's coma. We suggest that the swirls which we have mapped on the lunar surface are generally magnetised due to thermoremanence or shock remanence (or both) in a highly altered lunar regolith, which has not been sampled by any lunar mission.

The distribution of swirls also may provide a clue to the direction of impact. The two major concentrations of swirls seem to be nearly symmetric about a line of latitude, thereby suggesting an orbit close to the ecliptic. A highly inclined orbit could result in a more asymmetric distribution. The east-open, fan-shaped envelope of deposits and the possible remnant of one impacting nucleus (Goddard A) suggest that the Moon must

have been in the waxing phase. Moreover, the relative timing between Goddard A and formation of the swirls requires that the tail of the comet was pointing away from the surface. From these considerations, the proposed comet would have been an incoming, prograde body. Such an orbit would reduce the relative impact velocity since the comet and the Earth-Moon system would be travelling in the same direction.

The excellent preservation of all swirl deposits, which appear to be surficial in character, may indicate formation by a single cometary system. A simple impact by one body does not seem possible owing to the separate Goddard A and Ingenii swirl concentrations. However, these patterns could result from a split cometary body, a common phenomenon¹⁸. Although not essential to our argument, Reiner γ also could be included in this system if it formed $3\frac{1}{2}$ days in advance of the Ingenii/Goddard A patterns. Rotation of the Moon during this time would geometrically allow the formation of Reiner γ beyond 180° from Goddard A.

Thus, it is plausible that a prograde, incoming, split comet system impacting the Moon between a waxing crescent and gibbous phases. Goddard A may represent the impact crater by a nucleus, whereas a small crater on the rim of O'Day may represent a second nucleus. The Reiner γ swirls could be part of the latter event, rather than a separate nuclear region. Although the most visible results are the swirl patterns, the unusual dark-rayed/bright-rayed dimple craters and a large number of small bright-rayed (non-secondary) craters near Mare Marginis may indicate an additional flux of small-size (<10 m) debris impacting nearly simultaneously.

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Sequence of the human insulin gene

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The human insulin gene contains two intervening sequences, one is within the region transcribed into the 5'-untranslated segment of the mRNA and the other interrupts the C-peptide encoding region. A comparison of the human with the rat insulin genes indicates potential regulatory regions in the DNA segment preceding the gene and suggests that the ancestral form of the insulin gene had two intervening sequences.

DIABETES MELLITUS is a complex disease in which the primary clinical symptom is abnormally high fasting plasma glucose levels. In its many forms it may affect as much as 5% of the human population, and its incidence appears to be increasing¹.

The hormone insulin, isolated in 1922 by Banting and Best², regulates normal glucose homeostasis and also has other phy-

siological effects. Insulin appears to exert its biological activities through interaction with a receptor present on the membrane of most, if not all, cells³. This leads to a pleiotropic effect on several physiological processes including uptake of various nutrients, and enhanced synthesis of glycogen, lipids, certain amino acids and proteins⁴. In addition, it promotes proliferation of many

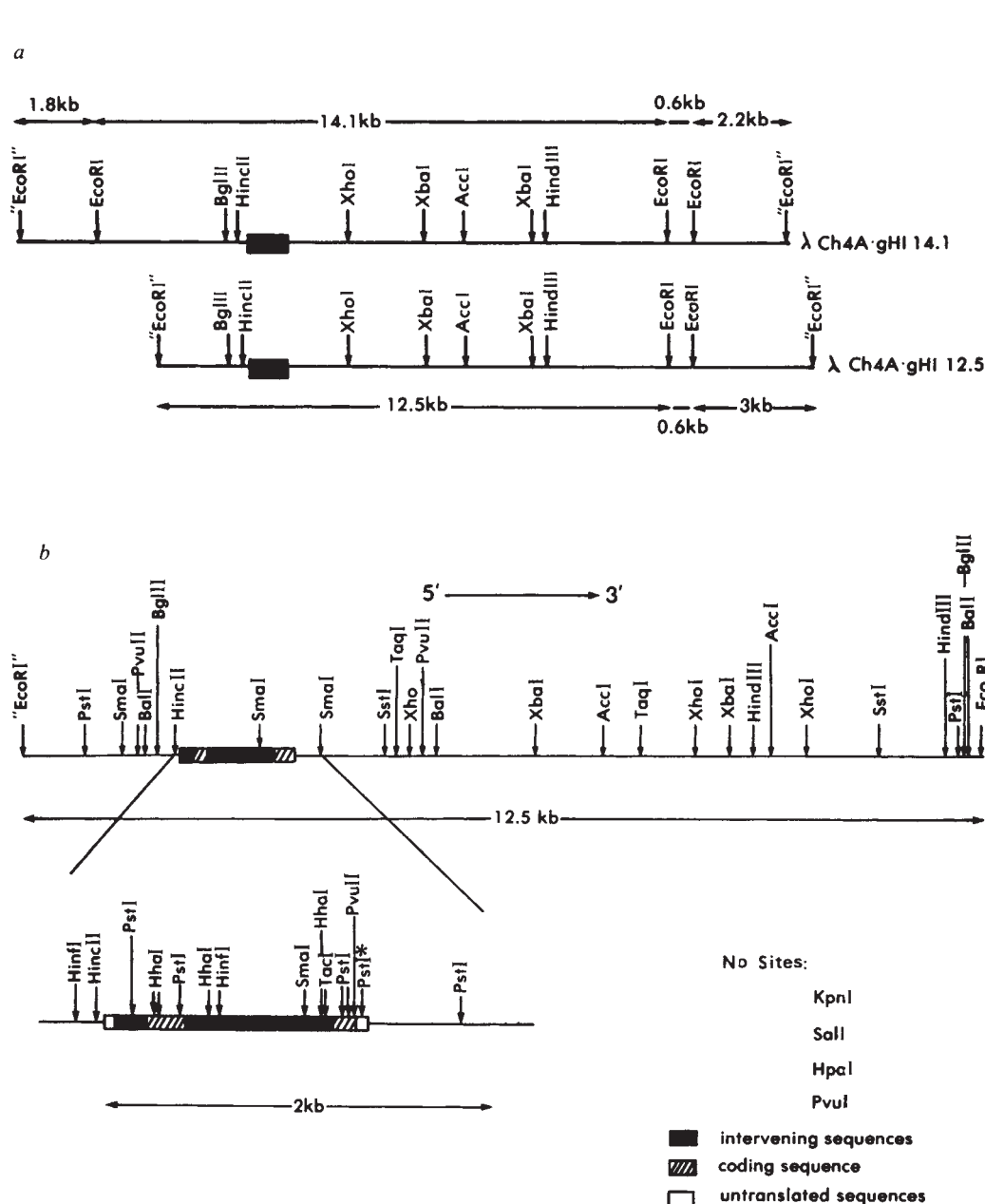


Fig. 1 a, Restriction maps of inserts of λ Charon 4A human insulin gene clones. A cloned library of approximately 20-kilobase fragments of human fetal liver DNA constructed as described by Lawn *et al.*²² was used. Plaques were screened for human insulin gene sequences as described previously¹⁹ by hybridisation with a nick-translated human insulin cDNA probe²¹. Phage containing the insulin gene were plaque purified and DNA was then prepared from phage grown in liquid culture. After digestion with *EcoRI* and agarose gel electrophoresis, the DNA fragments which contained insulin gene sequences were identified after hybridisation with human insulin cDNA²³. The order of the *EcoRI* fragments and the sites for other restriction enzymes within the human DNA inserts were determined by analysing multiple restriction enzyme digests¹⁹. The exact position of the insulin gene within the 12.5-kilobase (kb) *EcoRI* fragment of λ Charon 4A gHI 12.5, indicated by the box, was determined by more detailed restriction endonuclease mapping (b) and DNA sequencing. The position of the insulin gene in λ Charon 4A gHI 14.1 was inferred from the comparative restriction endonuclease analysis of the two clones. The *EcoRI* sites within quotation marks are those of the *EcoRI* linkers used in construction of the library and are adjacent to the λ arms. b, Partial restriction map of the human insulin gene and adjacent regions. The 12.5-kilobase insulin gene containing *EcoRI* fragment of λ Charon 4A gHI 12.5 was

subcloned into pBR322 (pGHI 12.5). The restriction sites were determined after single and multiple enzyme digests. The relative position of insulin coding sequences was determined by the hybridisation technique of Southern²³. The exact location and structure of the gene were determined by DNA sequencing. The orientation of the gene is indicated by the arrow.

animal cells in culture⁵. These processes may be mediated at least in part by an effect on the intracellular second messengers calcium and/or cyclic AMP⁶. Although administration of the hormone ameliorates the diabetic symptoms and allows a more or less normal life, diabetes and its complications are still the third leading cause of death in the US¹. The aetiology of diabetes is poorly understood; both environmental (dietary, viral and chemical) and genetic factors are involved^{1,7-9}.

Insulin has been isolated from a variety of vertebrate species^{10,11}. In all instances, the molecule is composed of two polypeptide chains (A and B) joined by disulphide linkages. In most species examined, there is only a single insulin and consequently, it is believed, a single gene. However, three species of rodents (laboratory rat, mouse, and spiny mouse) and two species of fish (tuna and toadfish) contain two distinct insulins^{11,12}, inferring the presence of two non-allelic insulin genes.

The immediate translation product of the insulin gene is a single polypeptide, preproinsulin, which contains a 'signal' peptide at the NH₂-terminus which facilitates the transit of the insulin precursor into the endoplasmic reticulum and is cleaved away during the process¹³⁻¹⁵. The resultant molecule, proinsulin, in which the peptides B and A are joined by the connecting C-peptide (NH₂-B-C-A-COOH), is the precursor of insulin¹⁶. The connecting peptide is believed to aid the formation of the appropriate conformation of the molecule and thus correct formation of the disulphide bridges. Consistent with this view, there is considerable variation in the structure of the C-peptide between species¹⁷.

Until recently there was no specific knowledge about insulin mRNA or the insulin gene. Using recombinant DNA methodology, we isolated a DNA complementary to the messenger RNA of rat insulin and determined its sequence¹⁸. The overall structure of rat preproinsulin was determined, and

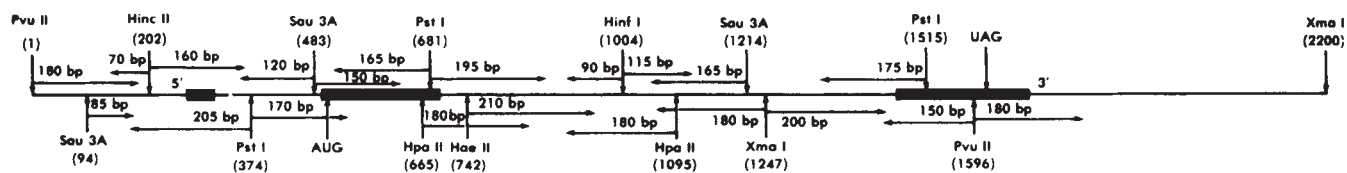


Fig. 2 Strategy for sequencing human insulin gene and adjacent regions. End (5')-labelled restriction fragments were sequenced by the procedure of Maxam and Gilbert²⁴. The cleavage products were separated by electrophoresis in 0.38-mm thick 10% and 15% polyacrylamide gels. The direction and number of bases determined from each labelled restriction site are indicated. The coding regions of the gene are indicated by the boxes. The orientation of the gene and positions of the translational start and stop are also indicated. The number in parentheses is the position of the restriction site (Fig. 3) used to initiate a particular sequencing run. bp, Base pairs.

in addition, certain unknown amino acid sequences were established. The cloned insulin cDNA was used as a hybridisation probe to isolate fragments of genomic DNA containing the insulin genes^{19,20}. The genes encoding rat insulins I and II each contain an intervening sequence of 119 base pairs in the 5'-untranslated portion of the mRNA. In addition the rat insulin II gene (but not the rat insulin I gene) contains an intervening sequence (499 base pairs) in the region of the DNA coding for the C-peptide²⁰. Again using the rat cDNA as a probe to screen bacterial colonies containing recombinant plasmids derived from human insulinoma mRNA, we isolated a cDNA clone encoding human preproinsulin²¹. The nucleotide sequence of the human insulin mRNA is quite similar to the rat insulin mRNA. The human insulin cDNA was used as a probe to screen in turn for insulin sequences in a library of human genomic DNA fragments. We report here the complete sequence of the human insulin gene and the immediately adjacent DNA areas, and compare it to the rat insulin I and II genes.

Isolation and restriction endonuclease digestion mapping

The human gene library in bacteriophage λ Charon 4A described by Lawn *et al.*²² was obtained from Dr T. Maniatis. The library was constructed by partial digestion of human fetal liver DNA with *Hae*III and *Alu*I restriction endonucleases, size fractionation, methylation with the *Eco*RI methylase, *Eco*RI oligonucleotide linker addition, and cloning in the λ vector, Charon 4A. We screened the recombinant phages for those containing human insulin gene sequences by hybridisation with a radiolabelled cloned cDNA fragment encoding human preproinsulin²¹. Two out of approximately 10^6 phages hybridised with the probe. These two clones were purified and the DNA characterised. One clone (gHI 12.5) contained *Eco*RI fragments of 0.6, 3.0 and 12.5 kilobases, the other (gHI 14.1) contained *Eco*RI fragments of 0.6, 1.8, 2.2 and 14.1 kilobases. The largest *Eco*RI fragment in each isolate hybridised with the cloned insulin cDNA. Since the library was constructed from human DNA fragments obtained by essentially random cleavage, the data suggested that the two clones represent overlapping fragments of human DNA. Further restriction mapping confirmed this view and allowed ordering of the *Eco*RI fragments (Fig. 1a).

The 12.5-kilobase *Eco*RI fragment of gHI 12.5 was subcloned in the plasmid pBR322. A detailed restriction map of this fragment was constructed (Fig. 1b). The restriction mapping experiments indicated that the gene was not co-linear with the mature mRNA, since the extent of the coding regions was greater than the 416 base pairs present in the cloned human insulin cDNA used as a probe. Furthermore, the enzyme *Sma*I generated two fragments of 900 and 1,500 base pairs which hybridised with the cDNA probe. Since no *Sma*I sites were predicted by the mRNA sequence²¹, it seemed likely that the gene possessed an intervening sequence containing an *Sma*I site. The pattern and sizes of the *Pst*I fragments hybridising with the insulin cDNA were also consistent with the presence of an intervening sequence within the gene (see later Fig. 5).

Sequence and general organisation

The complete nucleotide sequence of the cloned human insulin gene, gHI 12.5, was determined by the procedure of Maxam and Gilbert²⁴. The strategy used is presented in Fig. 2. Insofar as possible both strands were sequenced and all restriction

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CAGCTGTCGACGAGGACAGGCTCTGGCCACCGGGCCCTTCGTTAAGACTCAATGACCCGCTGGTCTCGAGG
100
AAGAGGTCCCGACGACCAAGGAGATCTCCACAGACCCAGCACCAGGAAATGGTCCGGAATTCGACGC
150
CTCAGCCCCCAGCCATCTGCCGACCCGCCACCCGCCCTAATGGCCAGGGCGAGGGGTGACAGGTAG
200
GGGAGATGGCTCTGAGAC(TATAAAG)CCAGCGGGGGCCAGCAGCCCTCAGCCCTCCAGGACAGGCTG
250
CATCAGAAAGAGGCCATCAAGCAG(GTCTGTTCAGAGGGCTTCGGTCAGGTCAGGTCAGGTTCCAGGG
300
TGGCTGGACCCAGGCCAGCCTCTGCAGCAGGAGGACCTGGCTGGCTGGCTGAGCATGTGGGGGTGAG
400
CCCAGGGGGCCCAAGGCAGGACCTGGCTTCAGCCCTGCCCTGCTGCTCCAG)ATCACTG
450
500 Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala
TCCTTCTGCC ATG GCC CTG TGG ATG CGC CTC CTG CCC CTG CTG GCG CTG CTG GCC
Leu Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly Ser
CTC TGG GGA CCT GAC CCA GCC GCA GCC TTT GTG AAC CAA CAC CTG TGC GGC TCA
His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr
CAC CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTC TAC ACA
Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln V
700
CCC AAG ACC CGC CGG GAG GCA GAG CAC CTC CAG C (GTGAGCCAAACCCCAATGCTGC
750
CCCTGGCGCCGCCAGCCAGCCCTGCTCTGGCTGCCACCCAGCATGGCGAGAAGGGGAGGAGGCT
800
GCCACCCAGCAGGGGGTCAGGTGCACTTTTAAAGAAAGATCTCTTGGTTCAGCTCCTAAAGTCACACG
850
CTCCCTGTGGCCAGTCAGAAATCTCAGCCTGAGGACGGTGTGGCTTGGCCAGCCCGAGATACATCAGAG
900
GGTGGGACCGCTCTCTCTCCACTGCCCCCTCAACAAATGCCCGGACGCCATTTCTCCACCTCATTTG
950
1000 ATGACCGCAGATTCAGTGTTTGTGTTAAGTAAAGTCTGGGTGACACAGGGGTACAGGGTGGCCACGGTG
1050
CCTGCTCTGGGCGAACCCCATCACGCCGAGGAGGGCTGGCTGCTGCTGAGCGGGCCAGACCCC
1100
1150 TGTGCCAGGCTCAGCGAGCTCCATAGTCAGGAGATGGGGAAGATGCTGGGACAGCCCTGGGGAGAG
1200
1250 TACTGGGATCAGCTGTTCAGGCTCCCATGTGACGCTGCCCCGGGGGGGGAAGGAGTGGACATGTGG
1300
GCCTTGGGGCTGTAGGTCCACACCCAGTGGGTGACCTCCCTTAACC TGGGTGACCCAGCCGGCTGGAG
1400
ATGCTGGGAGTGCACCTAGGGCTGGCGGGCAGCGGGGACGCTGTGCTGCTGCTGCTGCTGCTGCTG
1450
CCCCTGCTCCCTGCGGCTGTTCGGGAACCTGCTCTCGCGGACAGCTCTGGGAG) al Gly Gln Val
Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Gly Gly
GAG CTG GGC GGC GGC CCT GGT GCA GGC AGC CTG CAG CCC TTG GGC CTG GAG GGG
Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu
TCC CTG CAG AAG CGT GGC ATT GTG GAA CAA TGC TGT ACC AGC ATC TGC TCC CTC
Tyr Gln Leu Glu Asn Tyr Cys Asn AM
1650
TAC CAG CTG GAG AAC TAC TGC AAC TAG ACCGAGGCTTCAGGACGCCCAACACCGCCGCT
1700
CCAGCAGGAGAGATGAATAAAGCCCTTGAACAGC CTCTGCTGCCCCCTCTGTGCTGGGGGCCCT
1750
GGGCAAGCCCACTTCCCGGACCTGTGTGACCCCTCCACAGCTCTCTGACGCTCTCTGGTGGC

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Fig. 3 Sequence of human insulin gene and adjacent regions. The sequence of the human insulin gene and its translation into preproinsulin in one frame is presented. The 'Hogness' sequence, TATAAAG, is boxed. The putative 5'-end of the mRNA (capping site) is indicated by an asterisk, while the 3'-end of the mRNA (poly(A) addition site), is indicated by an arrow. The intervening sequences are indicated in parentheses and were positioned by comparison with the cDNA sequence of human insulin mRNA²¹ and the rat insulin genes^{19,20} and by using the GT/AG rule (see text). The last digit of the number is placed over the corresponding base.

The 416-base pair cloned cDNA of human insulin mRNA extends from 10 bases before the start of translation to the

The structure of the 5'-untranslated region of human insulin mRNA could not be determined from the human insulin cDNA clone since the clone lacks most of this region. However, a comparison of the DNA sequence preceding the human insulin gene with the same region in the rat insulin I and II genes should define the 5'-end of human insulin mRNA, as well as localising any intervening sequences in this region. The results of such a homology study between the rat and human genes (Fig. 4) reveal



Fig. 4 Homology of the human insulin gene with the rat insulin I and II genes. The human insulin gene sequence (gH) (Fig. 3) is compared with the sequence of the rat genes for insulin I (gRI)¹⁹ and insulin II (gRII)²⁰. 'Maximum' homology was achieved by suitable additions, indicated in the sequence by colons, and by using a computer homology program. The program allows editing and moving of sequences while comparing one against the other. The detection of important homologies is facilitated with the Maxalig subprogram which finds the 15 best alignments between the two sequences being compared, showing the homology score for each, the size of the largest common cluster (contiguous sequence of matches) and the location of such a cluster. Another feature enables the user to select subsequences and work with these independently of the parent sequences. This may be carried out to any desired level to set subsequences of subsequences, and so on. This was used to obtain maximum homology by comparison of similar functional regions, for example, IVS1 of human and rat. The upper, continuous sequence is that of the human gene. The lower, discontinuous sequence indicates the nature of the differences between the human and rat genes. The absence of a nucleotide in the lower sequence indicates that the human and rat sequences are the same; the presence of a colon in either sequence indicates a gap equal in length to the number of colons to be added to achieve maximum homology. The Hogness sequence, TATAAAG, is boxed. The positions of the putative 5' end (capping site; *cap) and 3' end (poly(A) addition site; arrow, poly(A)) of the mRNA are indicated. The intervening sequences are indicated within parentheses and the left and right borders marked IVS1 or 2, respectively. The occurrence of IVS2 between the first and second base of the valine codon at proinsulin amino acid position 39 is indicated by a 'V' at the left hand boundary and 'al' at the right. The initiation codon for preproinsulin is designated 'fMet'. The numbers in the left margin refer to the human sequence (numbered according to Fig. 3) and the rat I or II sequence (numbered according to refs 19 or 20, respectively). The upper panel presents the homology of the human insulin gene with the rat insulin I gene in the region before the first intervening sequence (IVS1), and the lower panel the entire homology of the human insulin gene with that of rat insulin II.

three regions of sequence homology in the 5' portion of the gene. The human and rat I genes (the rat II gene sequence does not extend this far²⁰) are homologous around nucleotide 186 of the human sequence (numbers are derived from the sequence in Fig. 3) and preceding a presumptive transcriptional initiation site. The second region (nucleotides 231–310) is centred around the 'Hogness sequence' TATAAAG (positions 233–239); no other TATAAAG-like sequence occurs in the 1,789 base pairs which have been sequenced. In the rat insulin genes, this region of homology includes the 5'-end (capping site) of the mRNA, the 43 bases corresponding to the 5'-untranslated portion of the

sequence is 43 base pairs after this A residue in the rat II gene. Taking into account the homology and applying the GT/AG rule, this segment of the human insulin gene is only 42 base pairs long. It therefore appears that a similar intervening sequence occurs in the human insulin gene with its boundary at guanosine 305 (G-305). The third region of homology in this section of the gene is centred at nucleotide 483, 17 base pairs before the ATG which encodes the fMet of preproinsulin. This undoubtedly corresponds to the 3'-boundary of the intervening sequence which began at G-305. Thus, the human insulin gene contains an intervening sequence of 179 base pairs in the DNA segment encoding the 5'-untranslated region of insulin mRNA; this is 60 base pairs longer than the 119-base pair intervening sequence in the rat insulin genes. In the human gene the 3'-border of this intervening sequence is 3 base pairs further from the fMet encoding ATG as compared to the corresponding sequence in the rat genes.

Thus the 5'-untranslated region of human insulin mRNA is 59 nucleotides; in rat insulin mRNA, it is 57. Both human and rat insulin genes contain an intervening sequence of different length in the DNA which encodes this portion of the mRNA, although the exact position from the boundaries of the leader segment are slightly different. The sequence reiterated at the boundaries is different between the two species as is the number of splicing frames (human: 4; rat: 5). As observed for IVS2 in the C-peptide region there is no obvious homology between this intervening DNA segment (IVS1) in the human and rat genes except at the boundaries.

In contrast to the substantial nucleotide sequence homology between the rat and human insulin genes evident in the 5' flanking region, the coding regions, and the 21 bases at the end of the 3'-untranslated region before the poly(A) site, there is no homology in the region extending further in the 3' direction.

Finally, the 1,789-base pair segment which contains the gene and adjacent regions is very GC-rich. The mean molar GC content of human DNA is 38.0% (ref. 26), whereas this segment has a content of 64.8%. The high GC content is uniformly present in all regions sequenced. The rat insulin genes also have a GC composition of 55%, which is greater than rat DNA, 40% (ref. 27).

Allelic variation

Restriction endonuclease analysis of the two human insulin gene isolates demonstrated a *Pst*I site in gHI 12.5 (Fig. 5, lane *b*) which is absent in gHI 14.1 (Fig. 5, lane *c*). Clone gHI 14.1 possesses a 585-base pair fragment instead of the 500- and 85-base pair fragments of gHI 12.5. The nucleotide sequence of the insulin gene of gHI 12.5 indicates that this additional *Pst*I site is centred at nucleotide 1,628 in the portion of the gene encoding the 3'-untranslated region of the mRNA. There are also two differences between the sequence of the human insulin cDNA clone, cHI-1 (ref. 21), and the genomic clone, gHI 12.5 in this region (Fig. 6); a C to T transition at nucleotide 1,628 which results in a *Pst*I site in gHI 12.5, and a C to A transversion at nucleotide 1,641. It is difficult to exclude the possibility that the differences observed in the cloned cDNA did not occur during cDNA synthesis. Nevertheless, the similarity in the restriction maps of the two genome isolates outside the insulin gene region (Fig. 1a) suggests that the observed differences reflect allelic variation. Furthermore, this interpretation indicates that the individual from whom the DNA was obtained for cloning was heterozygous since insulin sequencing as well as genetic studies²⁹ suggest only a single insulin gene per haploid genome complement.

Structural features

Present models of eukaryotic transcription propose that the primary transcript (pre-mRNA), which is co-linear with the DNA, is capped and polyadenylated before removal of any intervening sequences^{30,31}. Messenger RNA biosynthesis can be

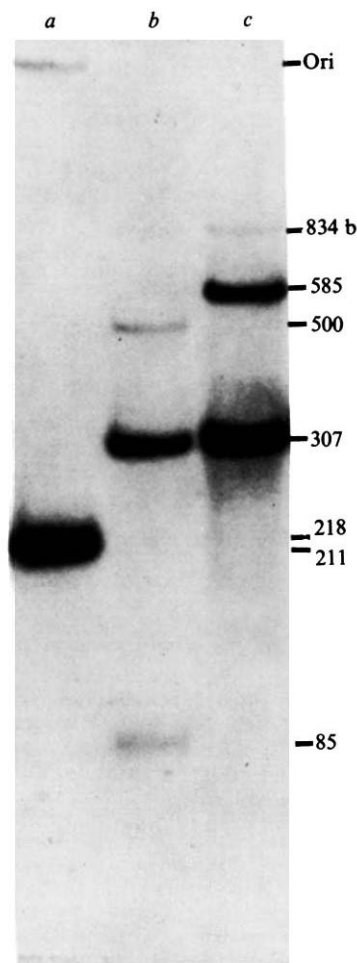


Fig. 5 Comparison of *Pst*I digestions of human insulin gene isolates gHI 12.5 and gHI 14.1. After *Pst*I digestion the DNA fragments were separated by electrophoresis in reversibly cross-linked 6% acrylamide–1% agarose gels²⁸. The fragments were transferred to nitrocellulose and hybridised with labelled human insulin cDNA. The size of hybridising fragments in base pairs are indicated. 'Ori' (origin) is the top of the gel. The lanes are *Pst*I digestions of *a*, human insulin cDNA clone, pCHI-1²¹; *b*, human insulin genome clone, gHI 12.5; *c*, human insulin genome clone, gHI 14.1. From the sequence (Fig. 3) and restriction mapping the order of *Pst*I fragments in lane *b* is (in base pairs) 5'–307, 834 (27) 85, 500–3'. The 27-base pair fragment is not seen as it migrated too rapidly.

mRNA as well as the 5'-boundary of intervening sequence 1 (IVS1). The extensive homology present in this segment (Fig. 4) suggests it contains the cognate region of the human insulin gene. Therefore, we tentatively assign the A at position 263 (Fig. 3) as the 5'-end of the mRNA. This is 24 base pairs after the end of the TATAAAG sequence. The start of the intervening

regulated at any of these steps. If the signals for these processes are dependent upon the primary structure of the DNA or RNA, the features common to both the human and rat insulin genes may indicate regulatory regions conserved during evolution.

As indicated in Fig. 4, there are two regions of sequence homology in the DNA segment preceding the sequence encoding the 5'-terminus of the mRNA. One region is around the sequence TATAAAG (G239), 24 base pairs before the start of transcription. This sequence is thought to interact with RNA polymerase to facilitate initiation of transcription³⁰. The other region is centred at A 184, 77 base pairs in front of the capping site. In the human but not the rat insulin gene this region is also part of a quite large imperfect palindrome (positions 155–213). A similar palindrome but of different sequence is present in mouse α -globin³² but not in the mouse β -globin genes^{33,34}. Since structures of this type in bacterial genes are often involved in the interaction with regulatory proteins³⁵, the human gene palindrome may also be a binding site for an insulin-specific regulatory protein.

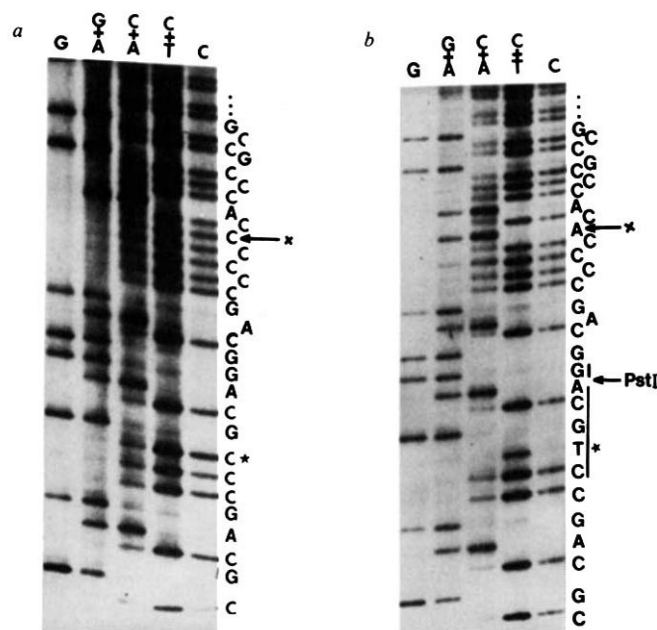


Fig. 6 Comparison of the sequence of the DNA region encoding the 3'-untranslated portion of insulin mRNA of gHI 12.5 (a) and cHI 1 (b). The sequence differences are indicated. The single *PvuII* site within the gene (gHI 12.5) or cDNA (cHI 1)²¹ was labelled with [γ -³²P]ATP and polynucleotide kinase. After secondary restriction enzyme digestion, the 5'-end-labelled fragments were sequenced by the procedure of Maxam and Gilbert²⁴. The chemical cleavage products were separated by electrophoresis in a 0.38-mm thick 15% polyacrylamide gel.

There are a number of direct repeat sequences located in the region immediately preceding the TATAAAG sequence. The sequences GGAAATT (positions 119–124 and 131–136), CC(G or C)ACCCC (positions 162–169 and 170–177), and GG(T or C)AGGGG (positions 195–202 and 209–216) each occur twice while the sequence CAGCC (positions 139–143, 145–149, and 152–156) occurs three times. Also, the sequence at the 5'-end of the mRNA, AGCCCTC (positions 263–269), is repeated immediately upstream at positions 256–262. The 5'-terminus of the silk fibroin mRNA is also repeated in a similar manner³⁶. Since most of these features are only characteristic of the human insulin gene and are not observed in the rat insulin genes, their physiological significance is not known. However,

since there are two insulin genes in rat and only one in human, some aspects of control may be different.

There is no obvious homology between the human and rat genes in the DNA sequence to the 3' side of the common 21-base pair sequence surrounding the AAUAAA sequence at the end of the insulin mRNA. Hence, if there are regulatory signals which occur 3' distal to this point, they are not a common feature of the insulin genes.

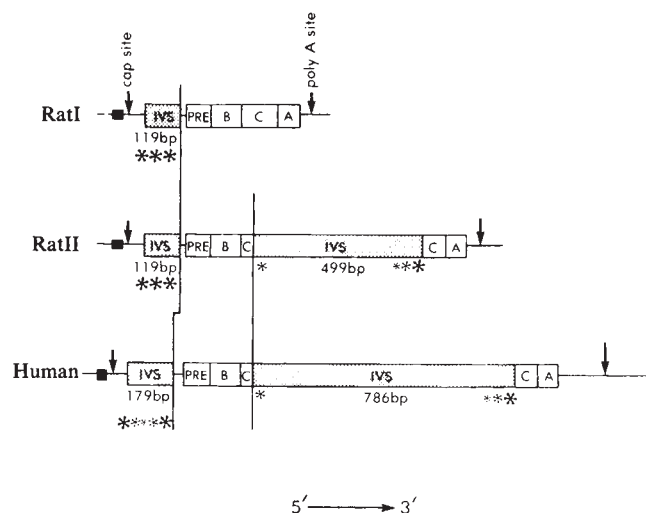


Fig. 7 Schematic comparison of human and rat insulin genes. The topology of the two rat insulin genes (I and II) and the single human insulin gene is displayed. The coding sequences for the peptide chains (pre-, B, C and A) of preproinsulin are represented by the clear boxes. Intervening sequences (IVS) are distinguished by the shaded areas with the length of each intervening sequence indicated below. The extent and position of nucleotide homology between intervening sequences of the two species is represented by the size of the asterisks—the larger the asterisk the greater the homology. Vertical lines describe the positions at which intervening sequences occur. The internal intervening sequence in the rat II and the human gene occurs in exactly the same position (valine 39 of the C-peptide region) whereas the position of the intervening sequence located in the 5'-untranslated region is displaced by three nucleotides in the human gene as compared with both rat genes. Also indicated are the sites for polyadenylation and capping (shown by arrows) as well as the Hogness box, a potential site for the initiation of transcription (indicated by the small black box). The arrow indicates the direction of transcription.

The signals in the nucleotide sequence which direct the capping and intervening sequence excision reactions are not obvious. However, as observed for other mRNAs²⁰, the 5'-untranslated region of the human insulin mRNA does possess some elements of secondary structure, whose possible regulatory role, if any, is undetermined. From a comparison of the similar regions of other genes, both the intervening sequences in the human insulin gene conform with the GT/AG rule proposed by Breathnach *et al.*²⁵. Also, as there are several ways in which each intervening sequence can be removed and still preserve the sequence of the mRNA (see ref. 37 for review), the structure(s) of the pre-mRNA molecule which affect intervening sequence removal remain elusive. The conservation of sequence homology around the boundaries of intervening sequences of the rat and human insulin genes suggest that the sequences of this region might be important. This homology is most extensive on the 5' side of the splice point, that is, before the GT at the 5' and the AG at the 3' borders of the intervening sequences. However, these features are not evident in comparisons between the rabbit and mouse β -globin genes³⁸.

Discussion

There now appear to be at least two types of mammalian insulin gene. The genes for human and rat II have two intervening sequences, while rat I has a single intervening sequence (Fig. 7). All three contain an intervening sequence in the DNA which encodes the 5'-untranslated or leader position of insulin mRNA. The position of this intervening sequence is the same in the rat I and II genes, but is displaced by 3 base pairs in the human gene. The human and rat II insulin genes contain an additional intervening sequence at exactly the same position in the C-peptide encoding region of the gene. The similarity in the structure of the human and the rat II insulin genes suggests that the ancestral mammalian insulin gene will contain two intervening sequences. It has been assumed that the human haploid genome contains a single insulin gene since only one insulin protein has been isolated. The two independent isolates of the human insulin gene described here have identical restriction endonuclease maps (Fig. 1a) in the region outside the gene. This is not usually the case for non-allelic genes, for example the rat I and II insulin genes^{19,20}. The two human insulin gene isolates, however, differ by at least one base change which results in the elimination of a *Pst*I site in the region corresponding to the 3'-untranslated portion of the mRNA. These data indicate that the clones represent alleles of a single insulin gene. Data from experiments on a mutant form of insulin which results in diabetes²⁹ as well as studies on familial hyperproinsulinaemia³⁹ are consistent with this hypothesis and furthermore indicate co-dominant expression of the two alleles of a diploid heterozygote. In rats and presumably in other species with two insulin genes, the single gene was duplicated and at least in rats has lost one of the intervening sequences. The mechanism of elimination of the intervening sequence from the rat I gene must have been precise since the amino acid sequence and nucleotide sequence around the splice have been conserved. Although the gene sequences which encode human and rat insulin mRNA and several sequences before the gene are highly conserved, the internal regions of the intervening sequences of these genes are highly divergent. In fact, so much so that it is difficult by homology studies to discern any meaningful regions of homology within the internal regions of the intervening sequence and therefore impossible to deduce anything about the evolution of these portions of the insulin gene or the cause of their different sizes.

Evidence from viral systems suggests that at least one intervening sequence in the mRNA precursor is necessary for normal mRNA accumulation⁴⁰. This would indicate a requirement for one intervening sequence in the insulin gene. The role of additional intervening sequences in insulin and other genes is unknown. In the case of rat insulins the additional sequence does

not substantially affect biosynthesis since both rat insulins are present in approximately equal proportions^{41,42}. It is interesting, however, that it is present in that region of the gene which encodes the most variable portion of the molecule (the C-peptide), yet Gln and Val adjacent to the splice have been highly conserved. Moreover, the region around the splice has been deleted in the insulin related proteins, insulin growth factors I and II and relaxin¹⁷. An examination of the genes for these proteins as well as the insulin genes of lower vertebrates might give us insight into the structure of the primordial gene.

The causes of diabetes are still poorly understood. The disease can be classified into insulin-dependent and -independent forms. The insulin-dependent form of the disease may result from a destruction of the insulin-producing β cells by chemicals or viral infection^{8,9}. However at least in one instance this form of the disease is the consequence of a mutated insulin²⁹. Its study by Tager *et al.*²⁹ established a genetic basis for this form of the disease in some individuals and, moreover, that both human insulin alleles are expressed. In the other, more common form of the disease, insulin-independent or maturity-onset, there is a pronounced correlation between family history and diabetes^{1,7}. Thus there can be a genetic component associated with both forms of the disease. The allelic differences observed in the 3'-untranslated region of human insulin mRNA are a form of polymorphism which is not manifested in the structure of the protein but could affect insulin biosynthesis. This form of allelism might be especially relevant if it occurred in functionally important regions of the gene or mRNA, for example, the DNA region around that encoding the 5'-end of the mRNA. Presumably in normal metabolic conditions, insulin biosynthesis might be unaffected, however under metabolic stress there could be a profound effect on insulin synthesis which could result in diabetes. This hypothesis can be tested by examining the insulin genes of diabetic individuals to determine if they possess alterations. Moreover the extensive nucleotide sequence conservation between human and rat insulin genes in regions outside those encoding preproinsulin immediately suggests those regions which should be examined for their possible role in the regulation of insulin biosynthesis. These analyses as well as surrogate genetic techniques, in which normal or *in vitro* mutated insulin genes are introduced into cultured cells or *Xenopus* oocytes, should increase our understanding of the genetic factors which regulate insulin biosynthesis.

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Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK

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In pancreatic islets a peptide corresponding to the C-terminal tetrapeptide amide of cholecystokinin and gastrin, Trp-Met-Asp-Phe-NH₂, is present in nerve terminals. This tetrapeptide amide is uniquely potent as a releaser of insulin and the other islet hormones, whereas larger cholecystokinins and gastrins as well as tetrapeptide analogues are considerably less potent. We suggest that neural release of the tetrapeptide amide is implicated in regulation of pancreatic hormone secretion.

HORMONAL peptides may also serve important roles as neuroregulators. The related gut hormones, gastrin and cholecystokinin (CCK), belong to this group of peptides. They are located in central¹⁻⁶ and peripheral nerves^{6,7}, synthesised in neuronal tissue⁸, and concentrated in synaptic vesicles from which they are released like other neurotransmitters⁹.

Recently we studied the distribution and molecular nature of CCK in peripheral nerves⁶, and in continuation of these studies we have observed CCK nerve terminals in pancreatic islets. The predominant form of CCK in these nerves seems to be the C-terminal tetrapeptide amide, Trp-Met-Asp-Phe-NH₂, common to CCK and gastrin, hereafter referred to as CCK-4. We have also examined the effect of various cholecystokinins and gastrins on the isolated, perfused pancreas and observed that CCK-4 was considerably more effective in releasing insulin than larger molecular forms of gastrin and CCK, confirming our earlier impressions in man¹⁰. The results suggest that the secretion of insulin and other islet hormones is under neural control by the tetrapeptide amide.

Structure of CCK and the application of antisera specific for different CCK sequences

CCK is closely related to gastrin both structurally and functionally. The hormones probably share a common evolutionary origin⁴. Their identical C-terminal sequence (Fig. 1) is responsible for their biological activity, and the remaining parts of the molecules modify their potency on different target tissues. Both hormones are synthesised and released as a range of molecules of different sizes and biological potencies^{2,11-16}. Because of this molecular heterogeneity and as the common sequence appears highly immunogenic, characterisation of CCK immunoreactivity by conventional radioimmunoassays and conventional immunocytochemistry is insufficient. We have, therefore, developed a range of antisera specific for different sequences of CCK-33 and gastrin-17 (Fig. 1); details of their production and characterisation have been described previously¹⁷⁻¹⁹. These antisera have proved valuable for the characterisation of CCK and gastrin cells^{4,19,20} and in identifying the molecular nature of gastrin and CCK in extracts of nervous tissue^{1,2} and in cerebrospinal fluid²¹. In the present study we have used the antisera

to examine the localisation and molecular nature of the pancreatic CCK/gastrin-like peptides. Moreover, we have studied the activity of different molecular forms of CCK and gastrin on pancreatic secretion.

Structure-activity relationship of the cholecystokinins and the islet-cell secretion

The effect of different molecular forms of CCK on the release of insulin and the other islet hormones was studied by perfusion of the isolated, porcine pancreas. The concentration of insulin, glucagon, somatostatin and pancreatic polypeptide in the effluent was determined by sensitive and specific radioimmunoassays, whose development and character have been described in detail previously²²⁻²⁵. We chose the porcine pancreas to study the effect of gut hormonal peptides²⁶, because the pig is the only species for which the structure of most gut hormones, including CCK, is known. The perfusion technique and the reliability of this *in vitro* model has been reported in detail previously^{26,27}.

The cholecystokinins stimulated the secretion of all pancreatic hormones in a dose-related manner (Fig. 2). CCK-4 was by far the most potent peptide. Even at the lowest concentration (10^{-10} M), CCK-4 was a powerful secretagogue. The intact tetrapeptide amide with free Trp- and -Phe-NH₂ termini seemed necessary for the activity. Thus, synthetic peptides with NH₂-terminal modifications, such as removal of tryptophan (CCK-3), extension by glycine (CCK-5, gastrin pentapeptide) or by BOC- β -alanine (Peptavlon) greatly reduced the secretagogue activity. C-terminal modifications also reduced the potency. Thus, the deamidated tetrapeptide, Trp-Met-Asp-Phe-OH, stimulated insulin secretion only at the highest concentration tested (10^{-6} M), and it was without effect on glucagon, somatostatin and pancreatic polypeptide secretion (Fig. 3).

The larger molecular forms, CCK-8 and CCK-33, were significantly less potent stimulators of the endocrine pancreas (Fig. 2). As the tetrapeptide amide constitutes the active site also of the gastrins, we compared the effect of CCK-4 with the

principal molecular form of the gastrin, the heptadecapeptide amide gastrin-17 which, likewise, was several-fold less potent than CCK-4 (Fig. 2). In contrast to their weak effects on the endocrine pancreas, the larger hormonal peptides (CCK-8, CCK-33 and gastrin-17) were all more effective than CCK-4 in stimulating exocrine, pancreatic secretion (Table 1). This observation is consistent with the absence of innervation of the exocrine pancreas by CCK nerves (Figs 4 and 5).

Innervation of the pancreatic islets by cholecystokinin nerves

The immunocytochemical studies were conducted on pancreatic tissues from pig, cat and hamster. Antisera specific for the C-terminus common to CCK and gastrin revealed immunoreactive nerves occurring in two distinct locations: islets and ganglia (Figs 4 and 5). These nerves enter the pancreas in large trunks which also contain numerous non-immunoreactive fibres. In contrast to the smooth fibres observed in nerve trunks, those occurring in and around ganglia and islets had a beaded appearance, suggestive of axon terminals with varicosities. The widespread distribution of immunoreactive nerves within the pancreatic islets and ganglia suggests a true innervation of the endocrine cell populations. The failure to detect immunoreactive nerve cell bodies in the pancreas, as well as the strong innervation of the ganglionic cells, suggests an extrinsic source for the nerves.

Our attempts to demonstrate the nerves with C-terminal-specific antisera were guided by previous observations on brain tissue showing that the CCK nerves contained a preponderance of a C-terminal tetrapeptide-like component^{2,6,28} which was detected only by these antisera⁶. The immunocytochemical results were corroborated by absorption controls showing that only peptides containing the C-terminal tetrapeptide amide sequence were able to inhibit the staining reaction (compare Fig.

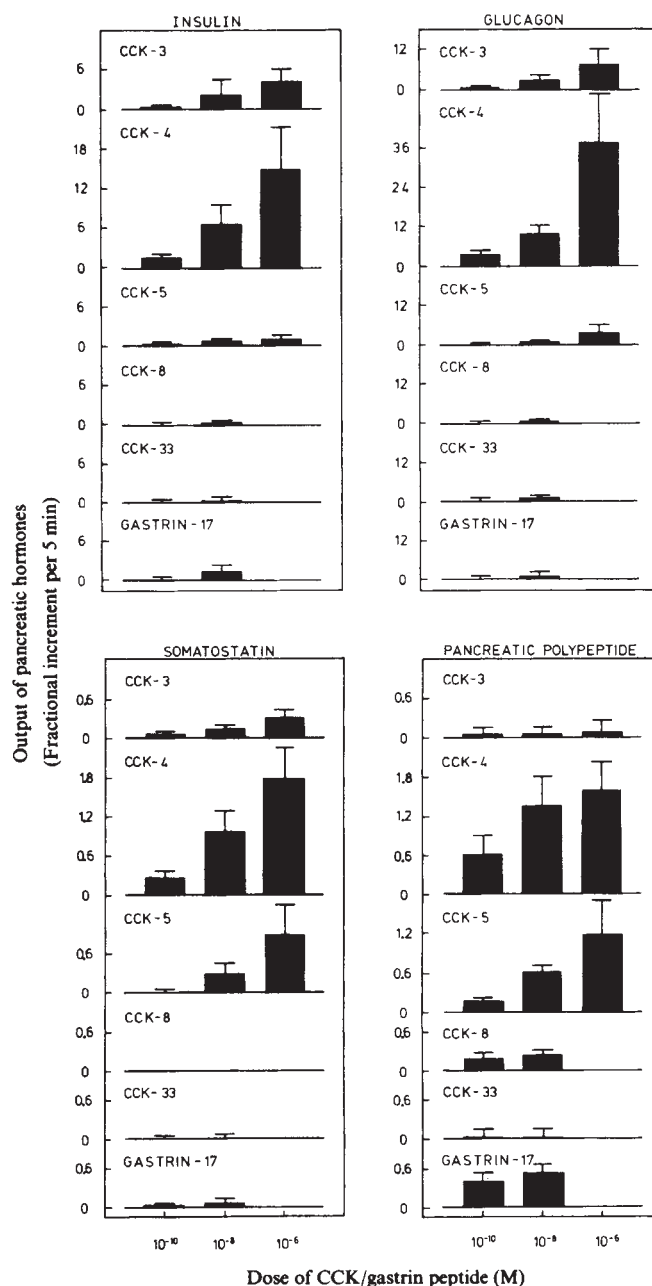


Fig. 2 Secretion of insulin, glucagon, somatostatin and pancreatic polypeptide from the isolated perfused, porcine pancreas in response to 5 min stimulations with CCK and gastrin peptides. The CCK/gastrin peptides were administered in concentrations of 10^{-10} , 10^{-8} and 10^{-6} M. The mean ($\pm$ s.e.m.) incremental hormone output ($\sim$ fold increase) above the output in the corresponding 5-min prestimulatory period was calculated. Each dose of each peptide was administered in seven perfusion experiments, except CCK-4, which was given during 11 perfusion experiments. CCK-3, -4, -5 and -8 correspond to the COOH-terminal tri-, tetra-, penta- and octapeptide amide of CCK-33. The preparation and technique for the perfusion experiments have been described in detail previously^{25,26}. In the present experiments the glucose concentration in the perfusate was 5–7 mM. The response of the four pancreatic hormones to stimulation with gastrin and CCK peptides showed only little dependency of the glucose concentration in the perfusion medium within the 5–7 mmol range. Studies of the effect of other large molecular forms of gastrin and CCK (gastrin-34, gastrin-14 and CCK-39) will be reported elsewhere (Jensen, S.L. *et al.*, in preparation).

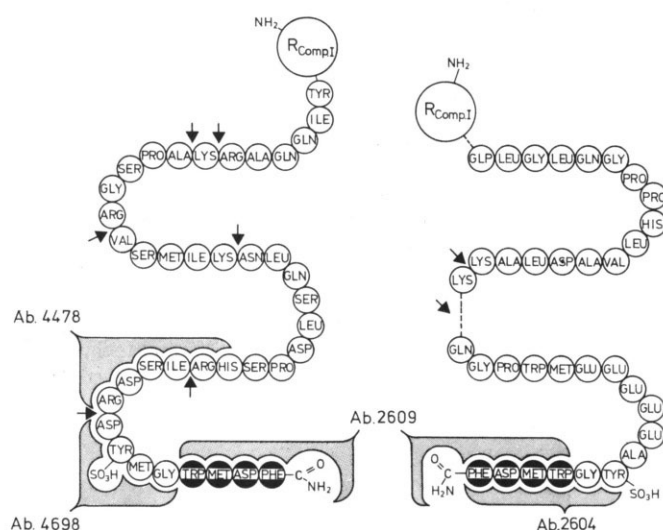


Fig. 1 The amino acid sequences of porcine cholecystokinin (left) and gastrin (right). The largest molecular form of CCK with known structure is a nonatriacontapeptide amide (CCK-39)^{36,37}. Molecular forms larger than CCK-39 have been found in nervous tissue and intestinal mucosa^{1-3,11}. These forms² have an additional extension at the NH₂-terminus of CCK-39. The amino acid sequence of the NH₂-terminal extension (R_{comp.1}) is not yet known. The largest molecular form of gastrin with known structure is a tetratriacontapeptide amide (gastrin-34)³⁸. A molecular form larger than gastrin-34, component I, has been found in serum¹⁴, gastrointestinal mucosa³⁹, the pituitary¹ and the vagal nerve⁷. Gastrin component I corresponds to gastrin-34 with an NH₂-terminal extension. The amino acid sequence of this extension, (R_{comp.1}) is not yet known. The shaded amino acid residues indicate the common C-terminal tetrapeptide amide sequence of CCK and gastrin. The arrows indicate points of tryptic cleavage. The brackets indicate the specificities of the antisera. Production and characterization of these antisera have been described previously^{2,18}.

4). Thus, a system of pancreatic nerves, storing either the free tetrapeptide amide or peptides containing this sequence, appear to innervate both the islet parenchyma and ganglia.

Attempts to localise the immunoreactive material at the ultrastructural level were unsuccessful. Embedding of formaldehyde-fixed tissue in resins or paraffin abolished immunoreactivity. Hence, post-embedding staining²⁹ was not possible.

Table 1 Exocrine secretion (ml h⁻¹, mean and range) from the isolated perfused porcine pancreas during stimulation with CCK/gastrin peptides

	Preperiod	10 ⁻¹⁰ M	Preperiod	10 ⁻⁸ M	n
CCK-4	0.15 (0–1.1)	0.1 (0–0.9)	0.1 (0–0.48)	1.2 (0.48–2.28)	11
CCK-8	0.1 (0.1–1.2)	1.5 (0.96–3.60)	0.2 (0.1–0.4)	6.0 (3.0–6.0)	7
CCK-33	0.1 (0–0.3)	2.2 (0.1–3.3)	0.6 (0–0.6)	6.0 (2.4–9.0)	8
Gastrin-17 (s)*	0.3 (0.2–0.9)	2.4 (1.0–4.8)	0.4 (0.1–1.3)	10.8 (5.0–14.1)	10

* (s), sulphated variant.

Instead we stained cryostat sections, which subsequently were embedded in Epon-812. Ultrathin sections revealed nerve terminals containing stained vesicles of varying diameters. The significance of this result is doubtful in view of the difficulties in carrying out appropriate controls on equivalent sections.

Apart from the nerves described above the pancreas also contains scattered endocrine cells displaying C-terminal gastrin-CCK immunoreactivity. Such cells were numerous in the feline

pancreas, scarce in the hamster pancreas and absent from the pig pancreas. As shown in a previous study, these immunoreactive cells also react with gastrin-specific, but not with CCK-specific antisera, and they are distinct from islet D (somatostatin) cells³⁰. In the feline pancreas, these cells occur in both ducts and islets, and are also scattered in the exocrine parenchyma. In the hamster, rare immunoreactive cells were confined to the ducts and exocrine parenchyma. Recent double staining experiments

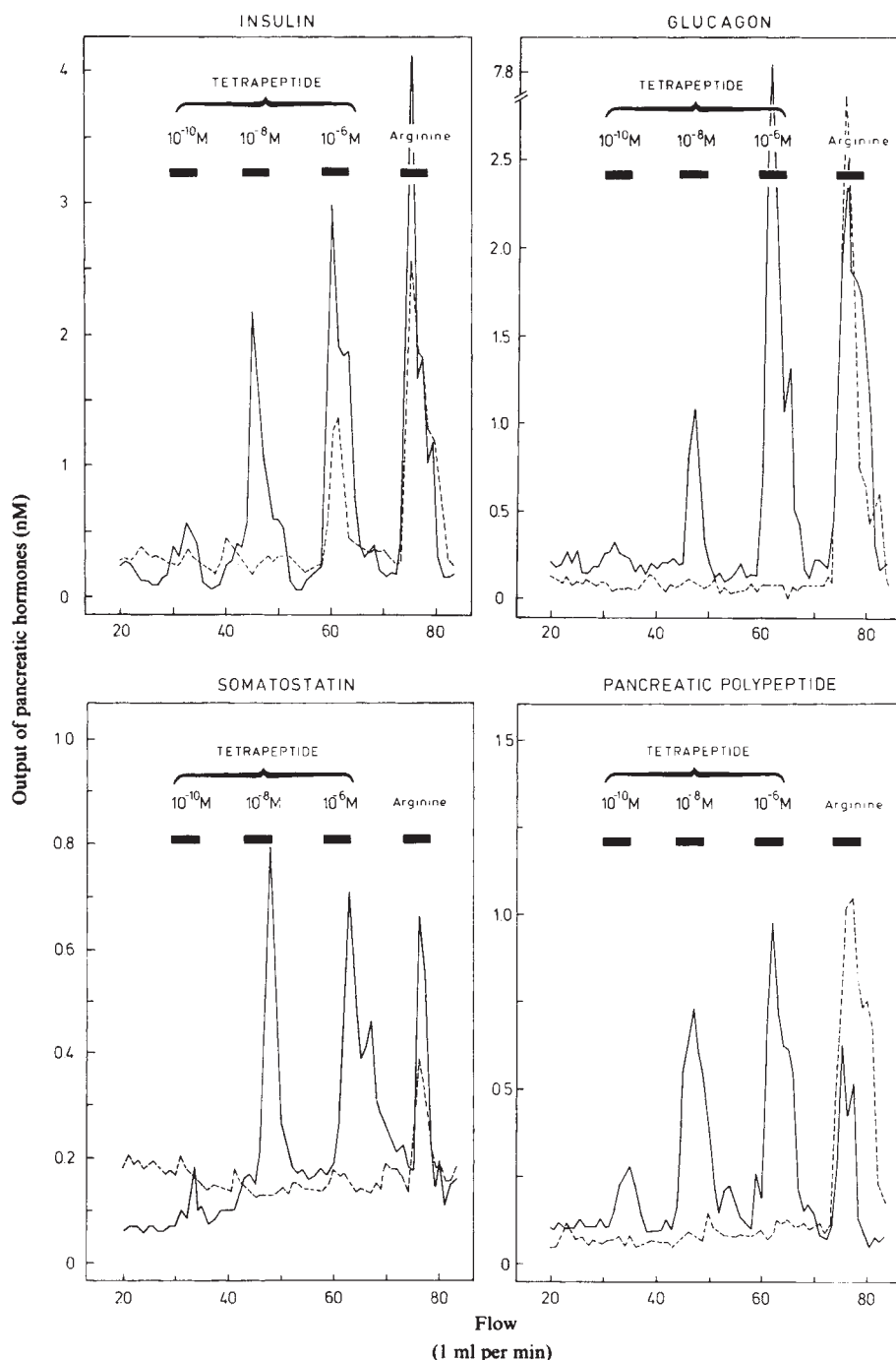


Fig. 3 Secretion of insulin, glucagon, somatostatin and pancreatic polypeptide from the isolated, perfused porcine pancreas in response to 5-min stimulations with the intact tetrapeptide amide (Trp-Met-Asp-Phe-NH₂; solid line) and with the deamidated tetrapeptide (Trp-Met-Asp-Phe-OH; broken line). Each line represents a typical perfusion experiment in which the tetrapeptide was administered in the three doses indicated. CCK-4 was given in 11 experiments (for mean output and s.e.m., see Fig. 2), and the deamidated tetrapeptide in four experiments, all with results as shown above. Each perfusion experiment was terminated by a 5-min stimulation with L-arginine (5 mM) to control optimal function of the preparation. For further details about technique, see refs 26 and 27.

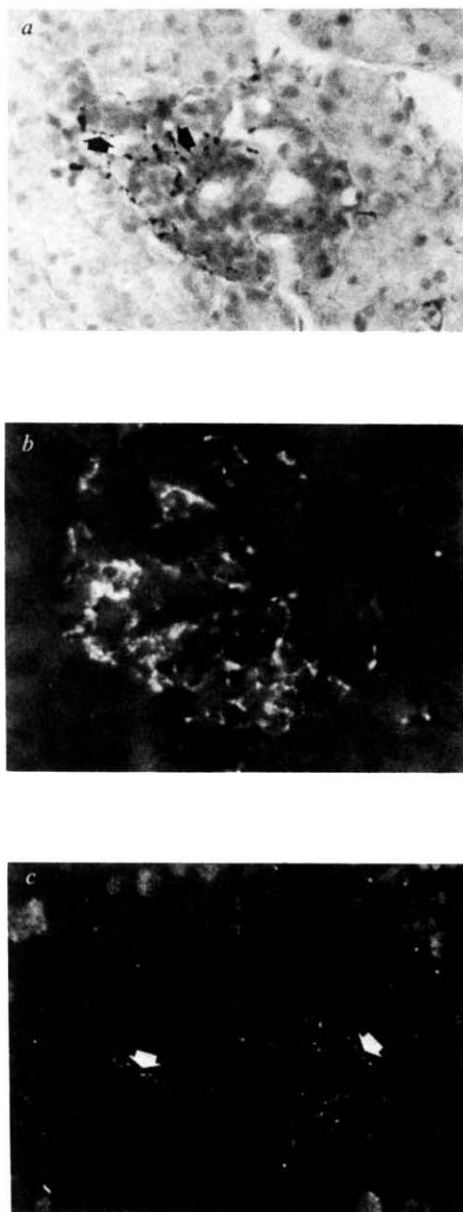


Fig. 4 Sections of cat pancreatic islets stained with antiserum 4562, recognising the common C-terminal tetrapeptide amide of cholecystokinin and gastrin, Trp-Met-Asp-Phe-NH₂. In *a*, the site of antigen-antibody reaction has been revealed with the peroxidase-antiperoxidase (PAP) method of Sternberger^{6,29}, whereas in *b* indirect immunofluorescence has been used ($\times 295$). Note the occurrence of numerous varicose immunoreactive nerve terminals, which surround the islet cells in an innervation-like pattern (arrows). Adult, freely fed cats ($n = 10$), hamsters ($n = 10$) and pigs ($n = 1$) were studied. The pig pancreas was perfused in situ with a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer pH 7.3, following cessation of stimulation experiments. Pancreatic slices were dissected out, postfixed in the same fixative for 1–2 h and rinsed in 20% sucrose in 0.1 M sodium phosphate buffer pH 7.3, overnight. Cats and hamsters were anaesthetised (mebumal/chloralose) and perfused via the heart with the formaldehyde solution. Following postfixation and rinsing in sucrose all pancreatic specimens were frozen in melting Freon 22 and sectioned in a cryostat. Immunocytochemical staining was carried out with antisera as described elsewhere⁶. The site of antigen-antibody reaction was revealed by immunofluorescence or by the peroxidase-antiperoxidase (PAP) procedure^{6,29}. Controls included conventional staining controls²⁹ as well as absorption controls including pretreatment of the antisera for 24 h at 4 °C with 100 μ g per ml diluted antiserum of *a*, pentagastrin (ICI); *b*, synthetic human gastrin I (ICI); *c*, synthetic caerulein (Farmatolia); and *d*, 99% pure porcine CCK-33 (V. Mutt). Absorptions against these peptides, but not against peptides lacking the common COOH-terminal tetrapeptide sequence (vasoactive intestinal polypeptide, substance P, ACTH, somatostatin) abolished staining. *c*, Section of hamster islet stained by indirect immunofluorescence as in *b*. Weak unspecific fluorescence (autofluorescence) is noted in the zymogen cells at the periphery of the islets. Unlike the fluorescence of the islet nerves, this fluorescence remains in control sections.

(for technical details, see ref. 31) in cat pancreas have now shown that the CCK nerves are primarily associated with glucagon cells in the islets.

The molecular nature of cholecystokinin in the pancreas

We extracted pancreatic specimens only from adult mammals in which we had ascertained that the immunoreactivity was located exclusively or almost exclusively in nerves, that is, hamsters and hogs. Porcine pancreatic tissue was obtained from a local abattoir 20 min post mortem. Hamster pancreas was dissected from anaesthetised animals. The tissue was immediately frozen at -70°C until extraction. Samples of frozen tissue weighing 0.5 to 2 g were immersed in boiling water (0.1 g ml^{-1} , pH 6.6), boiled for 20 min, homogenised and centrifuged ($2,000g$, 20 min). After decanting the first supernatant the pellet was extracted with 0.5 M acetic acid for a further 15 min at 4°C , followed by homogenisation and centrifugation ($2,000g$, 20 min). The water and acetic acid supernatants were applied to calibrated Sephadex columns (Fig. 6).

The chromatography, monitored by the different radioimmunoassays, disclosed the presence of five different molecular forms of CCK in the porcine pancreas (Fig. 6). The component with the largest molecular weight eluted in a position before CCK-39, and immediately after the void volume. The next three components eluted in positions corresponding to those of CCK-33, the C-terminal dodecapeptide of CCK (CCK-12), and the C-terminal octapeptide of CCK (CCK-8). Finally, a fifth component eluted at a position corresponding to the C-terminal tetrapeptide amide (CCK-4). When synthetic CCK-4 was used as standard for measurement of the fifth component, this component was seen to be far more abundant than the remaining CCK components (Fig. 6c). Apart from the immunoreactivity corresponding to CCK-4, specific gastrin immunoreactivity was not found in the porcine extracts. The nature of the different CCK-components was corroborated by re-chromatography on Sephadex G-50 and G-25 columns and by chromatography with a urea gradient. Using appropriate standards (CCK-33 for components I and II; CCK-8 for III and IV; and CCK-4 for V) the following concentrations of each component (I–V) were found: 1.2 ± 0.5 , 0.3 ± 0.1 , 1.5 ± 0.7 , 3.2 ± 1.1 and 19.6 ± 7.1 pmol per g porcine pancreas (mean $\pm$ s.e.m., $n = 4$). Extracts of pancreas for hamsters contained less immunoreactivity, but again the predominant component corresponded to CCK-4. An additional component eluting in a position corresponding to heptadecapeptide gastrin was assumed to have originated from the scarce endocrine gastrin cells in the hamster pancreas.

Several lines of evidence support the contention that the fifth component described above is identical to CCK-4: (1) The

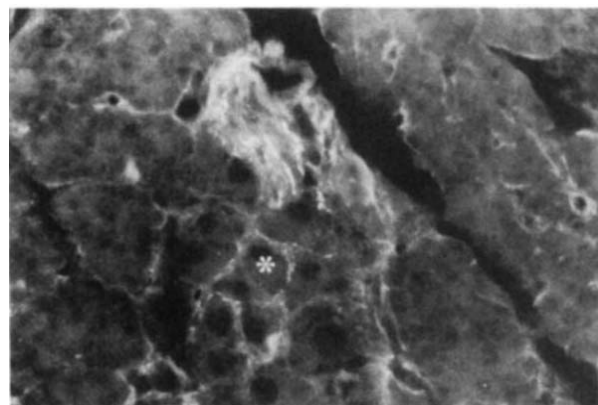


Fig. 5 Section of cat pancreas reacted with As 4562 in the indirect immunofluorescence procedure. Numerous preterminal nerves are seen to enter an intrapancreatic ganglion and to innervate non-immunoreactive ganglionic cell bodies (asterisk) ($\times 350$).

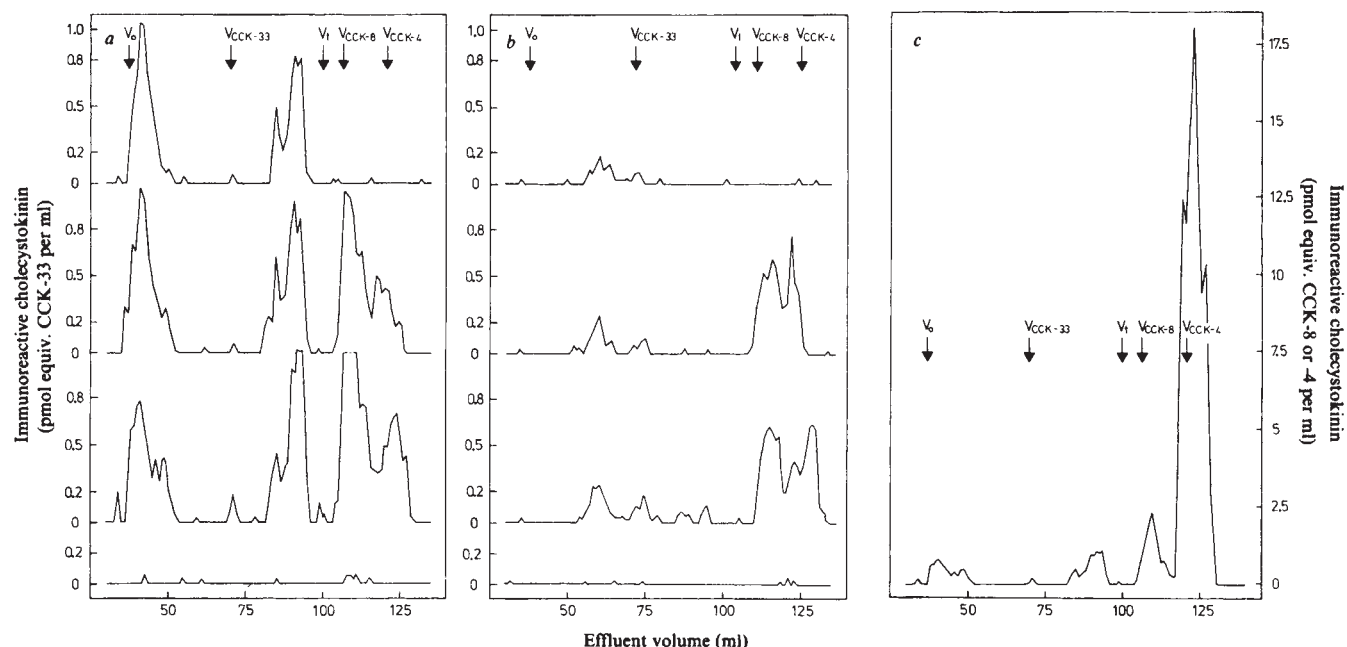


Fig. 6 Gel chromatography of extracts from the porcine pancreas, in which all cholecystokinin/gastrin immunoreactivity was confined to nerve fibres. Specimens of pancreatic tissue were obtained 20 min post mortem and immediately frozen. While frozen the tissue was cut in pieces weighing a few mg. The pieces were immersed in boiling water, for 20 min (0.1 g ml^{-1}), and subsequently homogenised for 5 min. After centrifugation, samples of the supernatant (panel a of the figure) were applied to calibrated Sephadex G-50 superfine columns ($10 \times 1,000 \text{ mm}$) eluted at 4°C with 0.02 M veronal buffer $\text{pH } 8.4$, at a flow rate of 4 ml per h . After decantation of the first supernatant, the pellet was extracted by 0.5 M acetic acid and rehomogenised for 5 min. After centrifugation, samples of the acid supernatant (panel b) was applied to columns calibrated and eluted as described above. The elutions of the boiled water extracts as well as the acetic acid extracts were monitored by four different radioimmunoassays, whose development and character has been described in detail elsewhere¹⁸. The first (upper) diagram in panel a and b illustrates the elution pattern as measured with CCK antiserum 4478, specific for sequence for 20–25 of CCK-33 (see Fig. 1). The second diagram in panels a and b illustrates the elution pattern using CCK-antiserum 4698, specific for sequence 25–30 of CCK-33 (see Fig. 1). The third diagram in panels a and b illustrates the elution pattern using gastrin antiserum 2609, specific for sequence 30–33 of CCK-33 (see Fig. 1). Monoiodinated ^{125}I -CCK-33 (ref. 18) was used as tracer and porcine CCK-33 as standard for the three mentioned antisera. The fourth (lower) diagram in panels a and b illustrates the elution pattern as measured with the gastrin-specific antiserum no. 2604 (ref. 17) used for further control of the specificity. Antiserum 2604 shows a negligible cross-reactivity with CCK when monoiodinated gastrin-17 was used as tracer⁴⁰. Synthetic human gastrin-17 was used as standard with antiserum 2604. Panel c illustrates the Sephadex G-50 elution pattern of the boiling water extract as monitored by the assay using antiserum 2609 and ^{125}I -CCK-33. Panel c thus corresponds to the third diagram in panel a, the difference being that synthetic CCK-4 was used as standard instead of CCK-33 for measurement of the immunoreactivity eluted in the position corresponding to that of CCK-4. Note the difference in order of concentration (1:5) on the ordinates of panel a and panel c. (For further details on the immunoreactivities of different molecular forms of CCK, see refs 18 and 28.)

component was detected only with gastrin antisera which are specific for the tetrapeptide amide sequence common to gastrin and CCK (Fig. 1, 4–6). These antisera are highly sensitive to modifications of the tetrapeptide amide structure. Deamidation, removal of NH_2 -terminal tryptophyl residue, exchanges or insertions of other amino acids almost abolish the antibody binding (unpublished results). Thus, a C-terminal tripeptide amide would not be detectable. (2) The component eluted in a position corresponding to that of synthetic CCK-4 in different gel chromatographic systems. (3) The extracts contained the complementary NH_2 -terminal fragment of CCK-8, detected by antiserum 4698, which is specific for the sequence¹⁸, Asp-Tyr(SO_3H)-Met-Gly (Fig. 1). The presence of a peptide resembling this NH_2 -terminal fragment suggests that the C-terminal peptide is produced by cleavage of CCK-8, and that it has a size corresponding to the tetrapeptide amide, as a larger C-terminal peptide, would require a smaller and hardly detectable NH_2 -terminal fragment.

The molecular forms of CCK in extracts of porcine pancreas correspond to the components in extracts of porcine nervous and intestinal tissue². The CCK-4-like component also predominates in these tissues^{28,32}. The predominance suggests that CCK-4 is the main product of the immunoreactive nerves in the pancreas. This accords with the demonstration of islet nerves with antisera specific for the C-terminus and with the weak and inconsistent staining of the islet nerves with antisera specific for other sequences of CCK-33. The larger components are presumably biosynthetic precursors in accordance with the biosynthetic relationship found for the different molecular forms of CCK in rat cerebral cortex^{8,33}.

Discussion

Our results suggest that CCK-4 (Trip-Met-Asp-Phe- NH_2) is present in CCK nerves in the pancreas and is involved in the regulation of islet cell functions. The present results, as well as those from previous studies in man¹⁰, show that CCK-4 has an immediate and potent effect on islet cell secretion.

The origin of the CCK-containing nerves in the pancreatic islets is unknown. However, CCK nerves are present in many regions of the body. Thus, we have reported that human and porcine brains contain CCK in quantities 10 to 100 times greater than those of other brain peptides^{1,2}; and that cerebral CCK is located in nerves in most regions of the central nervous system⁶. We have also found that the CCK in cerebral neurones fulfils several of the criteria for neurotransmitter function^{6,8,33}. In guinea pigs CCK is present in peripheral nerves which are particularly abundant in the muscular wall of colon⁶. We also observed a high density of CCK nerves in the coeliac-superior mesenteric ganglion⁶. The predominant molecular form of CCK in the peripheral nervous system corresponded mainly to CCK-4 (ref. 6). The pancreatic CCK nerves reported here correspond to the latter type.

Because of the high frequency of diabetes mellitus in man, the pancreatic CCK nerves are of considerable clinical interest. We have previously observed that CCK-4 is a potent insulin releaser in man¹⁰, suggesting that human islet cells may also possess receptors for CCK-4. As CCK-4-like peptides also occur in the human central nervous system and gut^{2,28,32}, it is likely that CCK-4 may also act as a neurotransmitter or neurohormone in the human pancreas. In view of the potent effect of CCK-4 on insulin secretion and possibly also on β -cell growth^{34,35} it may be

that CCK-4 or a suitable analogue may be of value in the treatment of insulin-dependent diabetes mellitus.

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LETTERS

The unusual supernova remnant CTB80

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The low latitude ($b = 2.8^\circ$) radio source CTB80 (ref. 1) is a probable, albeit unusual, supernova remnant² (SNR). A compact high brightness region with a very flat spectrum dominates its centre, and we find optical emission associated with this feature. Here we discuss evidence that CTB80 may be young, and in particular the possibility that it was observed in China at the beginning of the fifteenth century.

CTB80 is seen to best advantage in Fig. 1, a photographic representation of the 49-cm map made with the Westerbork Synthesis Radio Telescope (WSRT). Its radio emission is clearly the result of synchrotron radiation: the spectrum is nonthermal and large areas of the source are polarised. As unusual as the irregular morphology may seem, the most remarkable feature is the bright central peak with its flat radio spectrum. Notwithstanding an estimated spectral index $\alpha \approx 0$ between 49 and 6 cm, the fact that it is significantly polarised establishes its non-thermal nature.

We find that the nebulosity is most prominent on the E (or 'red') Palomar Sky Survey plate coinciding with the flat spectrum peak (Fig. 2). The agreement in both position and extent of the optical emission and flat spectrum radio component makes it highly probable that they are related. The only other nebulosity which might be associated with CTB80 is a narrow filament located some 10 arc min away from the prominent features, roughly along and aligned with the northern edge of the south-west radio ridge.

The lack of detailed radio-optical coincidence, which may be a feature of young SNR³, suggests that CTB80 could be of recent origin. Another, probably more direct indication of recent activity is the central flat spectrum component which with its morphology is somewhat reminiscent of the Crab Nebula⁴, or 3C58 (ref. 5). There have been several recent attempts^{6,7} to define and investigate the centre-filled or 'plethoric'⁸ SNRs as a separate class. Although CTB80 is more like G326.3 – 1.8 (ref. 9) than other members of this class, comprising as it does both compact, flat and extended, steep spectrum emission, it is perhaps worth considering whether it, too, might belong to the group. Weiler and Panagia⁷ argue that such SNRs are maintained for a relatively short period of time (10^4 yr) by a pulsar as in the Crab itself, after which their flat spectrum emission dies rapidly away. However, there does not seem to be a realistic pulsar candidate for CTB80: PSR1946+35 and 1952+29 lie too far away, about 3° north and south of it, respectively¹⁰, and a recent observation detected no scintillating component¹¹.

As far as the age of CTB80 is concerned, the purely morphological evidence is not decisive. While the irregular outer structure might suggest an old, well expanded object interacting with its environs, it could be argued that this is indicative of very recent activity, the form of the extended radio emission still dominated by the dynamics of the outburst which produced it. As noted above, the lack of corresponding optical nebulosity argues against an extremely old remnant with much swept-up material.

Table 1 Positions relevant to the new star of 1408

Object	α (1950.0)	δ (1950.0)
17 Cyg (south-east end of Niandao)	19 h 45 min	+33°37'
CTB80 nebulosity	19 51	+32 45
Cyg X-1	19 56	+35 04

But it is probably the compact, central component, whose flat spectrum suggests relative youth, which holds the key to this mysterious object. The fact that its spectrum shows no high frequency cut-off to 5 GHz enables us to set an age limit, depending on the magnetic field strength. For an equipartition field the value obtained, not greater than a few times 10^4 yr, is

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not especially valuable. If its age is 10^3 yr, then assuming that obscuration does not prevent our observing nebulosity near the centre of CTB80, there is a slight possibility that there is an historical record of the supernova. We have therefore examined a list of pre-telescopic supernova candidates compiled by Clark and Stephenson¹², and find that of their 75 events, only one is within 40° of CTB80.

Li Qi-bin¹³ has recently corrected the date of this guest star to 24 October 1408, shows that there may be nine Chinese references to it, and convincingly argues that it was a supernova. Li also suggests that it was the progenitor of the X-ray source (and black hole candidate) Cyg X-1, while we feel that an association with CTB80 is at least as likely. The only meaningful position, given in three of the chronicles, relates the new star to the Chinese constellation Niandao. This linear asterism runs from north-west to south-east, terminating at the latter end in 17 Cyg. A literal interpretation of the (translated) text, "to the southeast of Niandao", suggests a position both south and east of 17 Cyg (like that indicated by Clark and Stephenson¹²), consistent with CTB80 but excluding Cyg X-1 (Table 1). A looser interpretation of the wording would probably include anything within several degrees of 17 Cyg, and hence both candidate objects.

An indirect reason for preferring an association between CTB80 and the star of 1408 is that on both observational and theoretical grounds we expect supernovae to produce SNRs, and Cyg X-1 has no associated extended radio emission. One also wonders whether a supernova explosion would not have disrupted the binary system to which Cyg X-1 belongs. Although these arguments are somewhat weakened by the fact that we do not know what the event responsible for Cyg X-1 might have looked like, we will continue our discussion assuming that an association with CTB80 is more likely. What are the consequences of this assumption?

We concur with Li's conclusion that the star of 1408 must have been bright. (In addition to the reasons he gives, note that the description in the 'Ming Shilu' means that the star was probably observed just after sunset and before the end of

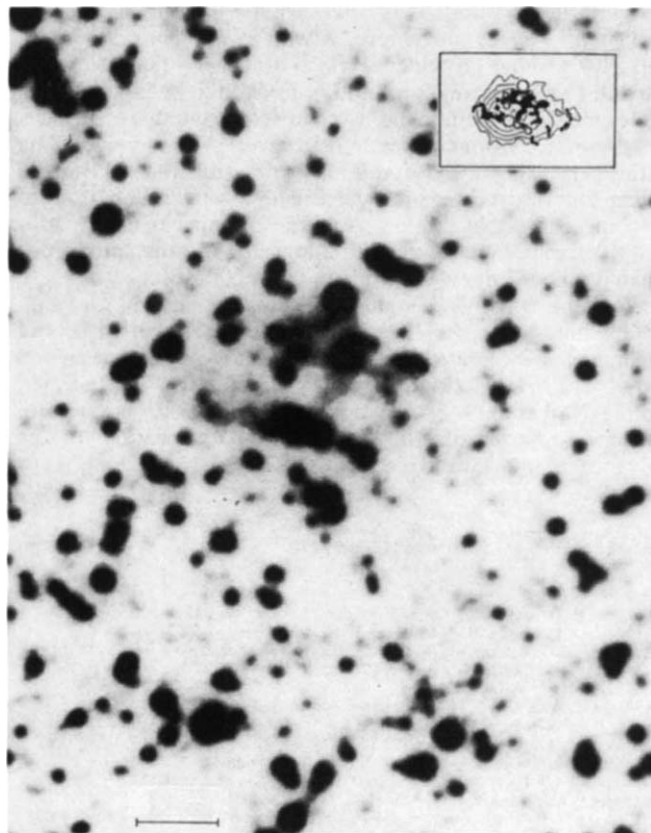


Fig. 2 An enlargement of the Palomar Sky Survey E plate (Copyright National Geographic Society-Palomar Sky Survey), showing the main nebulosity near the centre of CTB80. In the inset the optical emission has been sketched on a contour plot of the 6 cm map of the flat spectrum component made with a 7 by 13 arc s beam. The most prominent optical feature is the knot of emission near the western edge, elongated in position angle 80° . From prints made with various exposures it is clear that there are no sharp emission features and that although diffuse nebulosity probably fills the entire radio emitting region, there appears to be a hole in the centre. Scale bar, 20 arc s.

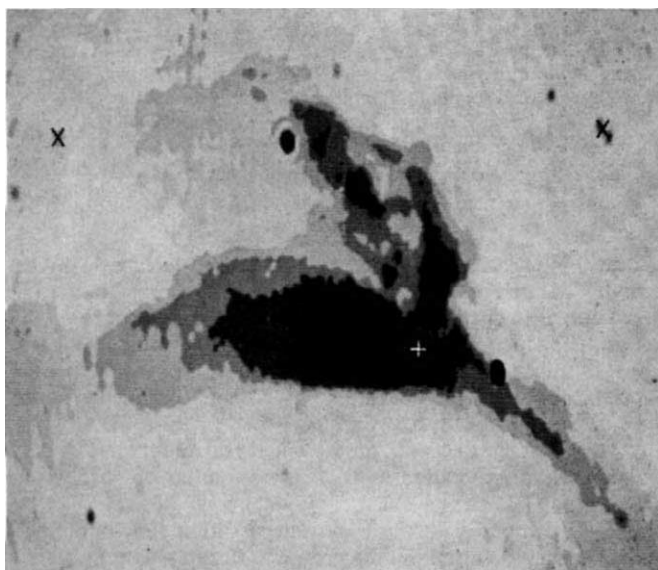


Fig. 1 A shaded contour photographic representation of the brightness distribution observed in CTB80 at 49 cm wavelength made with a 1 by 2 arc min beam. +, The highest peak, a flat spectrum compact component at the western end of the plateau; x, the positions of two strong sources which have been subtracted. North is at the top, and centre is $\alpha = 19^h 51.6$ min, $\delta = 32^\circ 49'$. Scale bar, 20 arc min.

astronomical twilight.) The chronicles consistently describe it as 'like a lamp', wording similar to that used for the supernova of 1572 ('large as a lamp') when its apparent magnitude was -3.5 mag (ref. 12). The lack of European reference to the 1408 star suggests that it was not as bright as the supernova of 1006 which did arouse interest¹². We conclude that the peak magnitude was probably -4 ± 1 mag. In a recent optical and radio investigation, Angerhofer *et al.*¹⁴ conclude that the likely distance to CTB80 is 3 ± 1 kpc, with at least 3–4 mag of absorption. If the maximum absolute magnitude of the supernova was -19 mag we would expect an apparent magnitude of -3.1 mag, in reasonable agreement with our above estimate for the 1408 star.

The spectral data obtained by Angerhofer *et al.*¹⁴ are consistent with a shell expanding at 35 km s^{-1} . If the distance (3 kpc) and age (570 yr) suggested above are correct, the shell has expanded at an average speed of 375 km s^{-1} . This is not irreconcilable with the spectral data if there has been substantial deceleration. Alternatively, the nebulosity may be excited circumstellar gas like the quasi-stationary flocculi in Cas A (ref 15).

Average velocities required in the outermost parts of the radio source are substantially higher, however. The optical wisps south-west of the main nebulosity must have travelled at some $13,000 \text{ km s}^{-1}$ since 1408, and the speed of material at the very edges of the radio emission would have to be at least four times higher. All of this assumes that a single outburst was the cause of CTB80 in its entirety, which may not be a necessary condition.

Apart from the possibility that we are seeing two totally unrelated sources juxtaposed (which we consider quite unlikely), we have no evidence which contradicts the notion that the outer arcs and ridges are the result of activity before 1408.

Despite the substantial body of data collected there is clearly much work to be done if we are to achieve a good understanding of this perplexing object. It does seem plausible that CTB80 is a young supernova remnant located within a few kiloparsecs of the Sun. Although it exhibits a superficial similarity to remnants like the Crab Nebula, its morphological and other properties make it unique among galactic nonthermal objects.

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Photoelectrochemical properties of metalloporphyrins

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The photovoltaic responses of *meso* tetraphenyl-, *meso* tetrapropyl- and octaethyl-porphyrins, porphines, chlorins, cofacial diporphyrins and mesoporphyrin IX diesters were investigated using two cell configurations:

Al|Porphyrin|Ag, and Al|Porphyrin|Fe(CN)₆³⁻, Fe(CN)₆⁴⁻|Pt.

We found (1) that the Al–porphyrin interface is photoactive: the action spectra closely follow the absorption spectra of the porphyrins, and this interface is best described as a semiconductor–insulator–metal diode consisting of porphyrin|Al₂O₃|Al; (2) that within a homologous series, in which the porphyrin skeleton is fixed but the metal is varied, the quantum yields parallel the ease of oxidation of the porphyrin in nonaqueous solvents. The more easily oxidised compounds exhibit the higher quantum yields; (3) no obvious correlations are found with the luminescent properties of the porphyrins in solution; (4) the morphology of the films influences the quantum yields: amorphous films are better than microcrystalline ones; and (5) the most efficient cells reach quantum yields of ~0.2 and energy efficiencies of ~1% for monochromatic light at the peak of the action spectrum in the region of 400–450 nm.

Although attempts to build photovoltaic and photoelectrochemical devices sensitised by dyes have met with varying degrees of success^{1–14}, the high quantum yields and energy efficiencies recently reported^{15–17} for the primary photoprocesses in photosynthetic organisms have stimulated a renewed interest in artificial systems based on chlorophyll derivatives. The inherent chemical fragility of chlorophyll and bacteriochlorophyll *in vitro* has focused attention on synthetic porphyrins, whose physical and chemical properties can be readily tailored by varying their metal and/or their organic framework. Examples of these are the recent reports of photovoltaic cells based on phthalocyanine^{18,19} and porphyrin²⁰ films sandwiched between metals of low work functions, such as aluminium or indium, and metals of high work function, silver or gold. These cells generate open circuit voltages (V_{oc}) of several tenths of a volt with short circuit currents of microamperes, and quantum

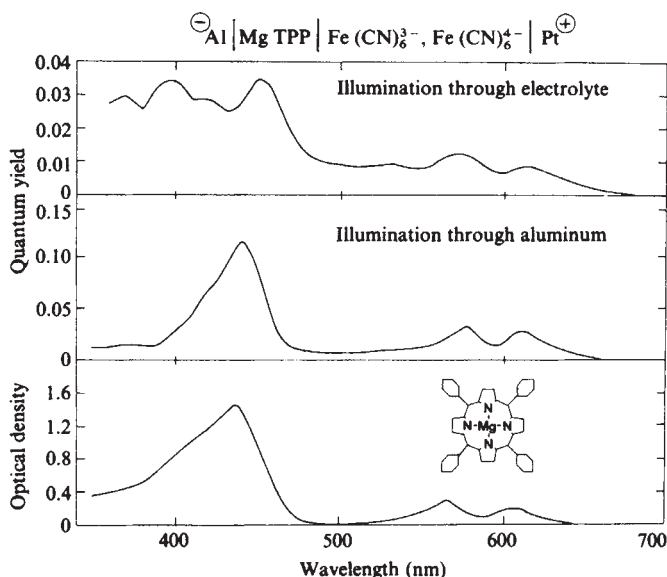


Fig. 1 Comparison of the optical spectrum of a magnesium tetraphenylporphyrin film with the action spectra, obtained for the cell shown, on illumination of the Al/porphyrin and Pt/electrolyte/porphyrin electrodes. Note the large differences in photocurrent quantum yields at the two interfaces.

yields as high as 0.1. The Al–dye (or In–dye) interfaces exhibit rectifying behaviours attributed to Schottky barriers induced by interaction of the p-type semiconductor dyes with the metals. A similar cell, which used zinc tetraphenylporphyrin layers on Al and a solution of ferri–ferro cyanide as ohmic contact, was reported by Wang²¹ to generate $V_{oc} > 1$ V and two such cells in series oxidised water to oxygen.

To assess the effects of metal and substituents, we have screened the photoresponses of a large number of porphyrin derivatives.

Figure 1 compares the photocurrent quantum yield obtained for a sublimed film of magnesium tetraphenylporphyrin (MgTPP) in a Wang cell with the optical spectrum of the porphyrin film. The large differences in photocurrent obtained on irradiation of the two electrodes and the similarity of the action spectrum at the Al electrode to the absorption spectrum of the porphyrin, clearly indicate that the Al–porphyrin interface is the photoactive region. Similar results are obtained with films deposited by rapid evaporation of concentrated solutions of dyes except that the absorption bands of the films are less diffuse and resemble solution spectra. Although the Al–porphyrin interface has been characterised²⁰ as a Schottky

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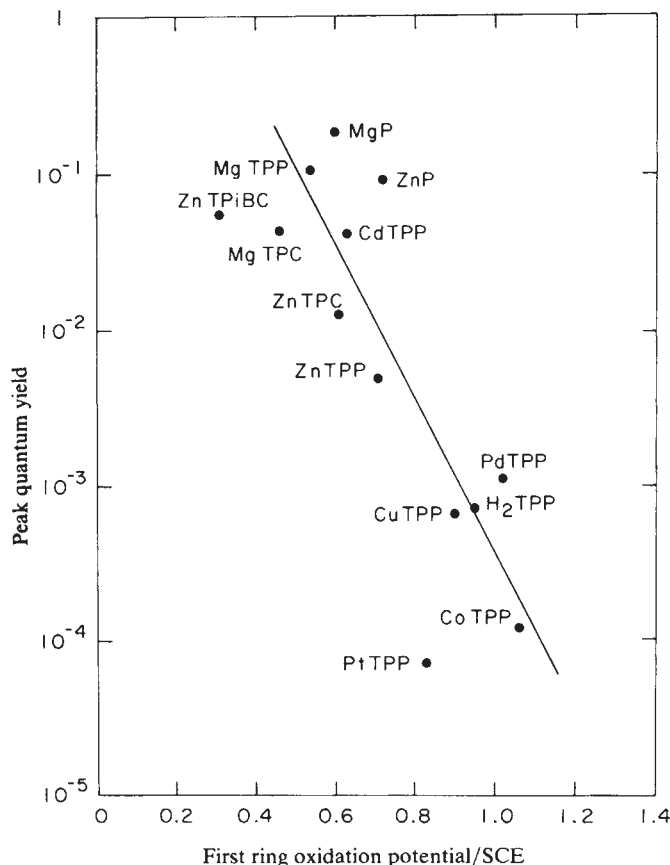


Fig. 3 Correlation between quantum yield and the first oxidation potential (versus SCE) of porphyrins in non-aqueous solvents. The line shown represents a least squares fit to the tetraphenylporphyrin data excluding PtTPP. (The latter is omitted because films of PtTPP on Al surfaces show unusually sharp absorption bands not observed on glass surfaces. A specific Pt-Al interaction may therefore account for the low quantum yield of PtTPP.)

The effects of film morphology, the use of mixtures of porphyrins, of other metals of low work function and of semiconductor electrodes are being investigated.

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Spatial distribution studies of thermoluminescence using a high-gain image intensifier

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Thermoluminescence (TL)—the light emitted when a previously irradiated material is heated—is normally studied by heating the material under a photomultiplier (PM). This measures the total light emitted within the acceptance angle of the PM, but provides no information on the spatial distribution of the TL. The dating of archaeological specimens by means of their naturally acquired TL is a common application of this method. While such a spatially-averaging technique is acceptable in studying TL from homogeneous materials, problems can arise when it is applied to inhomogeneous materials, such as certain natural calcites¹, where TL emissions may be correlated with internal irradiation dose rates. As we shall demonstrate, a high-gain image intensifier (IIT) can be successfully used to investigate the spatial distribution of TL from such materials at light levels where even fast photographic emulsions fail to record any emission. So far as we are aware this is the first time that high-gain image intensification techniques have been used in such studies.

The equipment, shown in Fig. 1, is centred on an EMI type 9914 intensifier. This is a magnetically-focused four-stage tube (of phosphor-photocathode sandwich construction) with a bi-alkali input and a P11 output phosphor. At an EHT setting of 40 kV the light gain of the tube is around 10^6 . This is sufficient to ensure that approximately 65% of the photoelectrons leaving

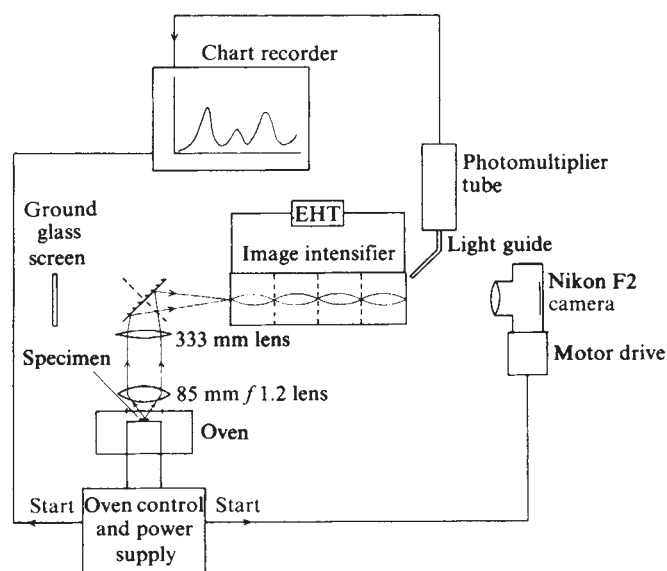


Fig. 1 Schematic diagram of the IIT and associated apparatus used to record the spatial distributions of TL emissions from a heated specimen in given temperature intervals together with the corresponding glow curve.

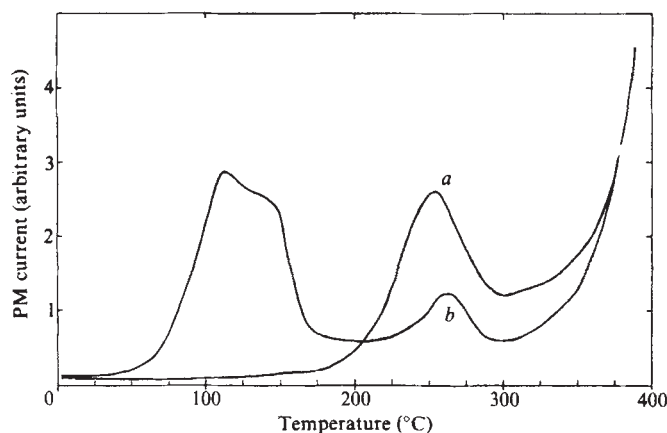


Fig. 2 Glow curves obtained from a sample of natural calcite obtained from a palaeolithic cave at Pont-Newydd, Wales. The specimen was heated linearly with time while a chart recorder traced the current from a photomultiplier tube which viewed the output phosphor of the IIT. Curve *a* shows the naturally acquired TL, and *b* the TL induced by an artificial γ irradiation of 12 krad.

the input photocathode (peak quantum efficiency 18%) are recorded as discrete 'points' on the film (Kodak Tri-X) taken in the camera viewing the output phosphor of the tube. (The camera is a motor-driven Nikon F2 with an *f*1.2 lens and a 4 dioptre achromatic doublet as a close-up lens.) The specimen is imaged onto the input photocathode using a microscope comprising a Canon 85 mm *f*1.2 photographic lens placed 'back-to-back' against a single-element lens of 333 mm focal

length, giving a linear magnification of 3.9. A rotating mirror allows the specimen to be brought into focus on either a ground glass viewing screen or the input photocathode. The advantages of such a microscope over a conventional one are the high aperture realised at low magnifications and the long working distance between the specimen and the 'objective' lens. The latter feature is important since the specimen is heated in an oven containing a 5-mm thick Spectrosil window and a 5-mm Chance-Pilkington HA-3 IR absorbing filter. The oven consists of an electrically-heated nichrome plate whose temperature, recorded by means of a NiCr/NiAl thermocouple, can be increased linearly with time². Starting the temperature ramp activates both the camera's motor drive and the time base of a chart recorder. The ordinate of the chart record shows the anode current of a PM viewing uniformly the total area of the output phosphor of the IIT; the resulting graph is conventionally called a glow curve.

In these studies we have used the intensifier system to observe the light emission from naturally occurring calcite of late Pleistocene age. Preliminary attempts to record even saturated TL in calcite using 3000 ASA film at 1:1 magnification had proved unsuccessful. Specimens were prepared in the form of 500 μ m thick slices. The surfaces were etched for 1 min in 1% acetic acid to eliminate spurious emission induced by the cutting of the slices³. Each specimen was first heated to observe its naturally acquired TL. The drained specimen was then given a γ irradiation of 12 krad and the artificially induced TL was observed in a second heating. Figure 2 shows the resulting glow curves for a sample of calcite (~10 mm across) obtained from a palaeolithic cave near Pont-Newydd in Wales. (The material from which the sample was prepared is stalagmitic, containing 1% of detritus, insoluble in HCl. Its mean ²³⁸U concentration is 0.5 p.p.m.) Figure 2*a* shows the natural TL and Fig. 2*b* the artificial TL. The

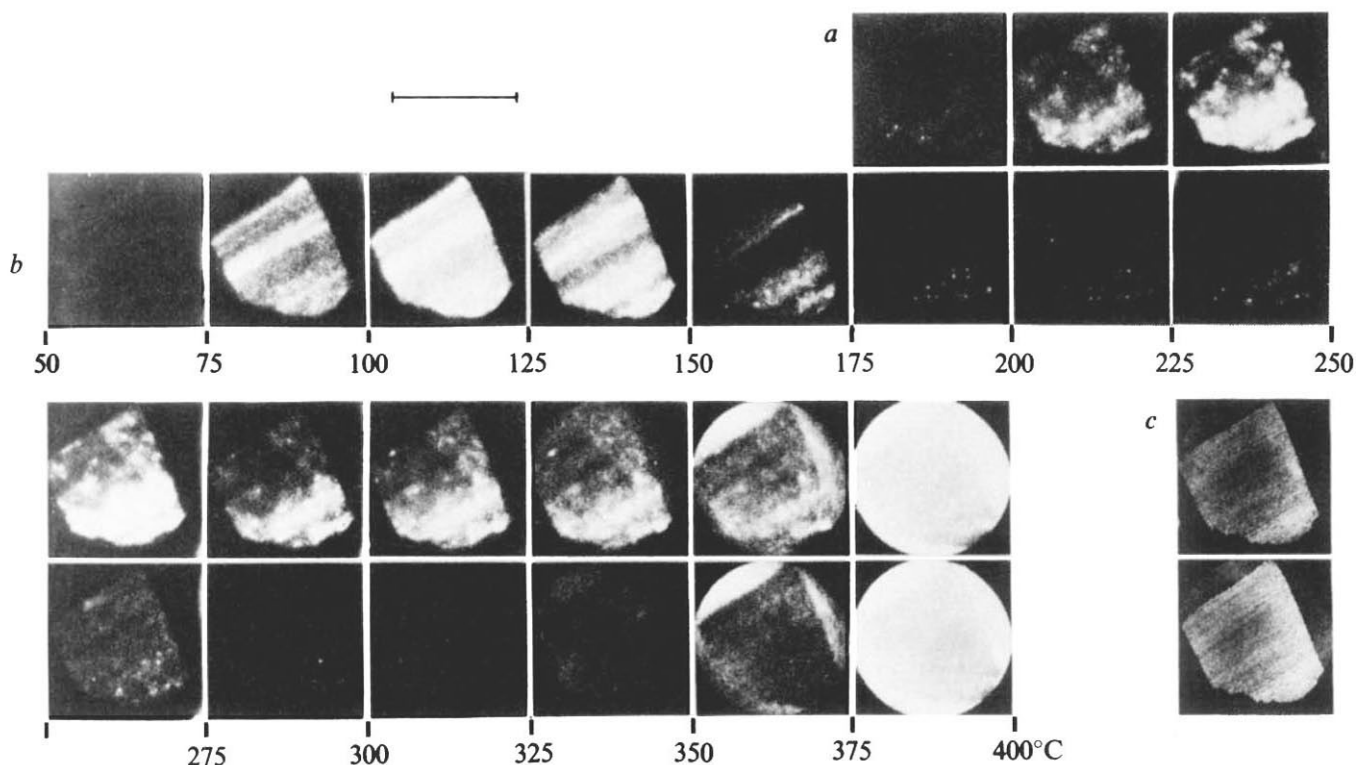


Fig. 3 Photographs of the output phosphor of the IIT showing spatial distributions of TL emissions from a specimen of natural calcite approximately 10 mm across on two subsequent heatings. Sequences *a* and *b* were taken concurrently with the respective glow curves *a* and *b* shown in Fig. 2. That is, *a* records the naturally acquired TL of the specimen, and *b* an artificially induced TL. Each frame was exposed for 10 s, and correspond to temperature intervals of 25 °C. (Frames in the sequence of natural TL below 200 °C are not reproduced as they recorded no light emission.) The 85-mm microscope lens was stopped at *f*1.2, and the Nikon camera lens at *f*1.2. The images recorded on the 35 mm Kodak Tri-X film were given standard development for a γ of 0.7 in Promicrol developer. All frames were given the same printing exposure. Photographs in *c* were taken at the end of each sequence of the cooled specimen under low-level illumination: a blue filter was used at the input end to enhance contrast. For these exposures both the microscope and camera lenses were stopped down to *f*5.6. Scale bar, 10 mm.

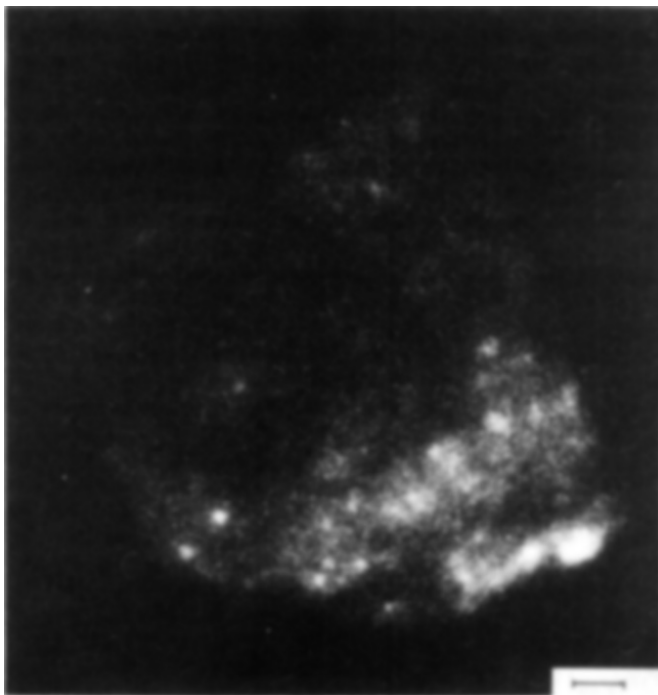


Fig. 4 Reproduction of the frame in sequence *a* of Fig. 3 corresponding to the temperature interval 275–300 °C, showing in more detail the intense 'spots' of TL emission against a more uniform, though banded, background. Scale bar, 1 mm.

corresponding sequence of photographs are shown in Fig. 3*a* and *b*. The temperature ramp rate was 2.5 °C per s and each frame was exposed for 10 s. Thus each frame records the integrated light emission within a 25 °C interval. Figure 3*c* shows the specimen photographed through the IIT under a low level of reflected light. (This procedure was adopted as conventional photomicrographs fail to reproduce geometrical distortions introduced by the IIT.) The glow curves shown in Fig. 2 are typical of those obtained by conventional PM techniques for many natural calcites. The peak at around 270 °C is commonly used in TL dating studies.

A striking feature of the sequences of photographs shown in Fig. 3 is the degree of non-uniformity in the light emission from both the natural and the artificial TL. (Frames in the sequence of natural TL below 200 °C are not reproduced as they recorded no light emission.) Looking at the sequence of natural TL emission (Fig. 3*a*) we see that most of the light emitted at the glow curve peak near 270 °C (Fig. 2*a*) in fact comes from localised 'spots' situated mainly in the lower half of the specimen. These spots may be seen more clearly in Fig. 4 which reproduces on a larger scale, the natural TL emission in the interval 275–300 °C. Their diameter is less than 0.5 mm and they appear against a more uniform emission. The glow curve of the artificial TL emission (Fig. 2*b*) shows a composite peak at around 125 °C in addition to the 270 °C peak. As can be seen from the sequence of photographs (Fig. 3*b*) the low-temperature emission is strongly banded with a few spots also contributing to the overall emission. Although the less intense emission at around 270 °C is more uniform it does show evidence of banding (particularly on the photographic negatives). The spots contribute a far smaller proportion of the high temperature emission in the artificial than in the natural TL. However, there is also evidence in the negatives of banding in the natural TL. Note that the growth layers visible in Fig. 3*c* are parallel to the banding seen in Fig. 3*a* and *b*. The bright background seen in the last two frames (375–400 °C, 400–425 °C) of each sequence is due to black-body emission from the heater plate.

There are at least two ready explanations for the 'spots' evident in the natural TL emission (Fig. 3*a*). The most likely one

is that they are associated with the detrital inclusions, insoluble in dilute HCl, which are present as 1% of the material. These mineral inclusions would be of geological age and likely to contain relatively high concentrations of uranium. Another possibility is that the spot emission originates from particles of limestone within the calcite matrix, the intensity of emission being mainly the result of their geological age. The apparent range of spot sizes (Fig. 4) is due to the varying depths of the emitting centres within the slice and the resulting variations in scatter of the emerging light. Observations on calcite samples from another site (Caune de l'Arago, France) have broadly confirmed the two basic features of spots and banding; the former being the predominant source of TL in young samples, the latter the predominant source in old samples (>200,000 yr). Adopting the methods of Fremlin and Srirath⁴ we have made fission-track maps of the old-age material and from these initial observations we have found that the uranium tends to be concentrated in the growth layers rich in iron impurities. These same impurity bands are also found to have higher optical attenuation coefficients. In addition the material's TL sensitivity to irradiation is believed to vary between the different growth layers. It is a combination of these three effects which produces the banding structure which we have observed in the natural TL, while the last two factors are relevant in the case of artificial TL. Further studies are in progress on the spatial distributions of the TL and uranium in calcite with the ultimate aim of improving the reliability and accuracy of TL dates. Although still at an early stage, image intensification techniques already seem to provide a powerful tool for the study of TL in inhomogeneous materials.

We thank Dr M. Aitken for suggesting that calcite be used in these studies, Dr S. Green and the National Museum of Wales for supplying samples, Professor H. Hopkins for advice in the design of the microscope and David Lusty for constructing the microscope. This work was supported by the SRC.

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Surface potentials of ice and thunderstorm charge separation

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There is no general agreement on the nature of the mechanism which causes thunderstorms to become electrified^{1,2}. Many scientists believe that charge is transferred when ice crystals collide with hailstones, and that subsequent gravitational separation results in field growth to breakdown magnitudes. Buser and Aufdermaur³ have proposed that the charging results from differences in the surface potential of ice. The measurements described here suggest that the surface potential of polycrystalline ice, such as would occur in a thunderstorm, is related simply to its past growth history. The sudden step in potential postulated by Buser and Aufdermaur as the ice surface changes from an evaporating to a growing state could not be detected. However, a natural hailstone grows by riming as it collects supercooled droplets, and these new measurements suggest that this effect results in much larger changes in surface potential.

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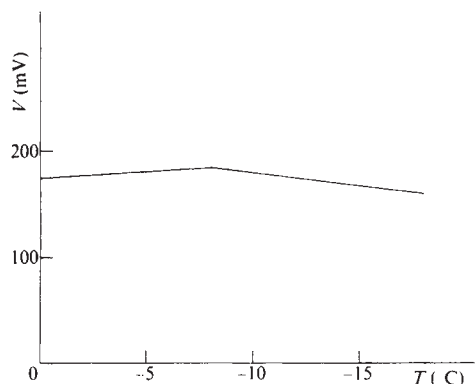


Fig. 1 Variation of the surface potential, V , of ice using gold electrodes with air temperature, T . The maximum potential always coincided with the ice temperature which was -8°C in this example; to the left the potential falls (ice cooler than air and condensing) and to the right it also falls as the warmer ice is evaporating.

Laboratory experiments which attempt to measure the charge separated when an ice crystal collides with a simulated hailstone, have not yielded consistent or predictable results. Possible clarification of this confusion is provided by Buser and Aufdermaur who found that when an ice crystal collided with various metal surfaces at -45°C the charge separated was proportional to the difference in the work function of the substances. Ice impacting on ice gave more variable results but if the target was evaporating it acquired negative charge whereas after deposition positive charge was favoured and the difference in charge per collision was equivalent to a change in the surface potential of the order of 0.2 V . This agreed with Takahashi's⁴ measurements of changes in the apparent surface potential of a copper electrode when ice was condensing on it or evaporating from it, and changes in the surface potential of a single crystal of ice also growing by deposition or evaporating at -10°C (ref. 5). The implicit assumption being that the surface potential change occurs in a single step. However, Buser and Aufdermaur concluded that the surface potential can be modified by so many factors that it may be difficult to predict in natural situations.

We report measurements of changes in the surface potential of ice made using a vibrating capacitor technique similar to that of Takahashi⁵. A cylinder of polycrystalline ice 10 mm thick and 50 mm diameter formed from distilled water was attached to the lower noble metal electrode by heating the electrode to near 0°C and warming the surface of the ice with hot air. The lower electrode was mounted on a Peltier element so that the specimen could be warmer or cooler than the ambient air. The upper electrode was made of gold plated gauze to allow an equilibrium vapour flux to the surface; if a solid electrode was used the results were variable and dependent on ventilation.

Figure 1 shows the uniform change in surface potential of about $1.5\text{ mV } ^\circ\text{C}^{-1}$ as the temperature of the air was varied whilst keeping the ice and metal substrate at a constant temperature. The change in the surface as it evaporated or grew by deposition was clearly visible to the eye, but was not associated with any sudden change in potential. The upper electrode was always kept 1°C warmer than the lower one to prevent frost deposit. Without this precaution potential changes showing hysteresis similar to those of Takahashi⁵ were obtained as frost formed on the gauze and then evaporated.

Similar results to Fig. 1 were obtained for different ice specimens at different temperatures and dew points and for electrodes of Au, Pt, or Pd. Changing the lower electrode altered the voltage offset but not the shape of the curves. The measurements were reversible with no hysteresis. We confirmed Hobb's⁶ statement that copper-ice electrodes give results which are unpredictable and not reproducible, but we could produce results comparable with Takahashi's⁴ if we prepared the copper electrodes in the exact manner he describes.

The most striking changes in surface potential were observed when the ice surface was rimed with supercooled droplets from an ultrasonic generator (radius $50\text{ }\mu\text{m}$, water content $1\text{--}2\text{ g m}^{-3}$) falling at terminal velocity. Figure 2 shows that the change in surface potential was a function of temperature. The ice, air and supercooled water droplets were all at the same temperature; although a subsidiary experiment showed that if the ice was a few degrees warmer than the air the same results were obtained. Identical results were obtained with $10\text{-}\mu\text{m}$ radius droplets.

If the ice was only partially covered with frozen droplets a smaller change was observed, but when further layers were added to an already complete layer no further increase in potential resulted. The potential change was reversible. If the rime on the surface of the ice was melted and then allowed to refreeze slowly, the potential returned to its original value. As soon as the melted surface was refrozen the process could be repeated with a new layer of droplets. The slow refreezing of the surface was achieved by heating the upper electrode while it was close to the ice. If a very thin surface layer of water was rapidly frozen by exposing it suddenly to a stream of cold air the same change was observed as with riming.

The changes in potential could be very persistent. If the rime was left for 24 h the potential was practically unchanged and surface melting returned it to the previous day's value. Care was taken not to confine the ice specimen mechanically around its perimeter, otherwise slow and unpredictable changes occurred as the temperature varied. This is probably due to the stress within the ice⁷. If the rimed surface of the ice was exposed to an electrical field of 500 kV m^{-1} , or a charged probe touched the surface, the potential returned to its previous value a few seconds after the field was removed.

The effect of impurities was found to be small: 10^{-4} M solutions of NaCl, NH_4OH , and HF gave the same results as Fig. 2 and rainwater gave values within 20% . These changes persisted for many hours. When the concentrations were increased 100-fold, changes were more variable and the voltage decay took only several minutes. These higher concentrations are quite unrepresentative of atmospheric conditions.

Note that during the actual freezing of the droplets of both distilled and doped water large transient potentials are developed which we interpret as Workman-Reynolds freezing potentials⁸. Because the chance of an ice crystal impacting on a hailstone at a position where a supercooled droplet is in the process of freezing is so small, these freezing potentials are not likely to be important in thunderstorms.

Previous measurements^{5,9,10} of changes in potential during riming showed a steady change in potential proportional to the riming rate, and it was postulated that charged splinters were ejected, but it is now established¹¹ that the splinter production was caused by the high concentration of CO_2 used for cooling.

The origin of this potential change during the rapid freezing of supercooled water is a formidable theoretical problem. The

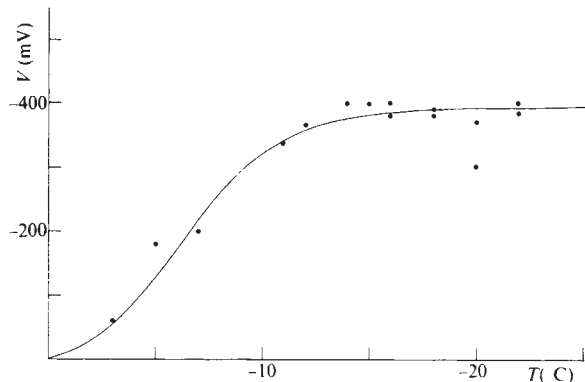


Fig. 2 Change in the surface potential, V , of ice after riming as a function of temperature, T . The unrimed ice surface is assigned a potential of 0 V .

temperature dependence in Fig. 2 and the fact that rapid freezing of a very thin layer of surface water gives identical results, suggest that the rate of freezing is a controlling parameter. The freezing velocity of ice in supercooled water varies with the square of the degree of supercooling¹² which is similar in form to the first part of Fig. 2.

When ice is subject to thermal stress so that cracks appear, potentials up to 40V have been measured⁷ which decay rapidly and in a temperature dependent manner. The potentials developed in these experiments resulting from the rapid freezing of supercooled water were two orders of magnitude less and only decayed rapidly if the ice was heavily doped or subject to external stress.

The large differences in surface potential between a rimed and a non-rimed ice particle should be a more powerful mechanism for separating charge when ice crystals collide with hailstones in thunderstorms than the much smaller potentials existing between evaporating and condensing ice surfaces.

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Allophane isolated from a podsol developed on a non-vitric parent material

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The relationship between allophane, an X-ray amorphous, short-range ordered aluminosilicate gel, found in soils derived from volcanic ash (Andosols) and the poorly ordered clay minerals present in the subsoils of podsoles (Spodosols) developed on non-vitric parent materials is uncertain. The similarity of many of the physical and chemical properties of these soils has led to postulations that 'allophane-like' materials also occur in podsoles^{1–4}. The high chemical reactivity of allophane and its intimate association with both soil organic matter and crystalline phyllosilicates and oxides has meant that earlier studies have involved either the examination of mixed crystalline-microcrystalline clay systems, or the use of dispersing reagents that would have caused at least the partial solution of any poorly ordered phases present. The established instrumental techniques of differential thermal analysis and IR absorption spectroscopy may be used to characterise allophanic materials, but only when they constitute virtually the whole of the clay fraction⁵. The present study characterises an allophane sample successfully isolated from a podsol profile.

The close similarity of the properties of naturally occurring soil allophanes and synthetic aluminosilicate gels^{6,7} suggests that the former are likely to have formed by precipitation from solution. If such a solution mechanism is involved, a vitric parent material should not be an essential requirement for allophane formation. The dominance of vitric parent materials in studies

involving soil allophanes, and the comparative paucity of convincing observation of allophane derivation from other parent materials⁸, may simply reflect the difficulties involved in isolating and characterising allophane when it constitutes only a small part of a clay fraction.

An opportunity to isolate and hence characterise a poorly ordered aluminosilicate gel formed during the genesis of a podsol has arisen during the study of a catena of podsolised yellow-brown earth soils on Bealey Spur, in the eastern foothills of the Southern Alps, North Canterbury, South Island, New Zealand (43°02' south latitude, 171°37' east longitude). The parent materials of the soils are mixed colluvium and locally derived loess overlying glacial till. The parent materials are all derived from rocks of the Canterbury Suite⁹. The dominant lithotype in the suite is a thick-bedded sandstone of Upper Triassic age. There are smaller occurrences of siltstones, mudstones, conglomerate, stratiform volcanics (basalts and dolerites), limestone and banded chert. The soils have developed under a forest vegetation of mountain beech (*Nothofagus cliffortioides* var. *solandri*).

Table 1 Chemical properties of the clay-sized separates of a podsol and selected allophanes

Size fraction (µm)	Podsol <0.2	Podsol 0.2–2	Taupo B <0.2	Taupo C <0.2
Ignition loss 105–600 °C (%)	47.3	43.6	43.9	27.7
Organic carbon (%)	15.3	14.2	13.2	6.3
Total Si (%)	13.9	16.7	17.5	21.6
Total Al (%)	32.4	28.1	25.4	21.7
Total Fe (%)	1.2	1.9	7.9	6.0
SiO ₂ /Al ₂ O ₃ mole ratio	0.82	1.1	1.3	1.9
Oxalate-Si as % total Si	67	39	28	14
Oxalate-Al as % total Al	94	90	87	71
Oxalate-Fe as % total Fe	41	19	92	78
SiO ₂ /Al ₂ O ₃ mole ratio (extracted)	0.58	0.47	0.44	0.38
NaOH-Si as % total Si	76	57	76	68
NaOH-Al as % total Al	98	90	90	79
SiO ₂ /Al ₂ O ₃ mole ratio (extracted)	0.64	0.72	1.1	1.6
OH ⁻ released in 25 min by 0.85 M NaF at pH 6.8 (mmol g ⁻¹)	39.8	32.9	23.9	22.2

Results are expressed on an ignited mass basis except for ignition loss and organic carbon which are on an oven-dry basis.

X-ray and microscopic examination of the sand fractions of the catena soils show them to be composed largely of quartz, altered feldspars and rock fragments consisting of quartz particles in a strongly weathered matrix. Minor to trace amounts of muscovite, biotite, chlorite, hornblende and garnet occur, particularly in the less weathered lower horizons. No fragments of volcanic glass could be detected.

The only minerals that could be determined in the silt fractions of the catena soils were quartz, feldspar, mica and chlorite. The clay fractions were dominated by phyllosilicate minerals derived from mica and contained minor amounts of gibbsite, kaolinite, allophane and quartz. Thermal and selective dissolution analyses indicated that allophane occurred widely throughout the catena. The greatest amounts of allophane and of gibbsite occurred in the lower part of the B horizons.

In the toeslope position of the catena the loess and colluvium were underlain by sand and gravel-sized sediments of similar mineralogical composition. These were thickly coated with a gel material. The advantage of the toeslope position is that translocation of the gel into an horizon that is essentially free of other clay-sized components has facilitated its isolation.

Fine ($<0.2\ \mu\text{m}$) and coarse ($0.2\text{--}2\ \mu\text{m}$) clay fractions of water-dispersed gel were separated by sedimentation and analysed by a range of instrumental and chemical techniques. In Table 1, and in Figs 1 and 2 the results are compared with data obtained from allophane isolated by water dispersion from the B and C horizons of a vitric Andosol, the Taupo sandy silt, from the Taupo Volcanic Zone of the central North Island¹⁰, and with that given by a synthetic aluminosilicate gel⁶ containing 60% by mass of Al_2O_3 .

Examination of the fine and coarse clay separates from the podsol by X-ray diffraction established their poorly ordered nature. X-ray diffraction patterns showed a broad hump in the baseline extending from 2.5 to 5.0 Å. The coarse clay fraction alone gave a weak diffraction peak at 3.35 Å which indicated the presence of a small amount of quartz. No evidence for the presence of cristobalite, feldspar, gibbsite, imogolite or phyllosilicates could be obtained from this technique or from thermal analysis, transmission electron microscopy or IR spectroscopy.

The IR spectra obtained from the podsol separates closely resemble that obtained from the synthetic gel (Fig. 1). Absorption maxima at 980, 550 and $350\ \text{cm}^{-1}$ reflect a very low degree of silica polymerisation, and a corresponding dominance of Al—O , AlOOH and Al—O—Si bonds. The distinct shoulder at $1,080\ \text{cm}^{-1}$ in the coarse clay spectrum indicates the presence of a silica-rich component in that fraction, but the absence of absorption at $800\ \text{cm}^{-1}$ argues against the presence of appreciable amounts of discrete silica. The Taupo allophanes on the other hand have been shown to be mixtures of a highly aluminous gel phase similar to that found in the podsol or prepared synthetically, and a more siliceous comminuted volcanic glass phase¹⁰.

Molar $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratios, based on total elemental composition, are lower for the podsol gels than for the Taupo allophanes (Table 1). As the latter are known to be mixtures of a gel phase and a comminuted glass phase, selective dissolution of the gel was attempted using both acid ammonium oxalate and sodium hydroxide as extractants^{11,12}. Both reagents extracted an alu-

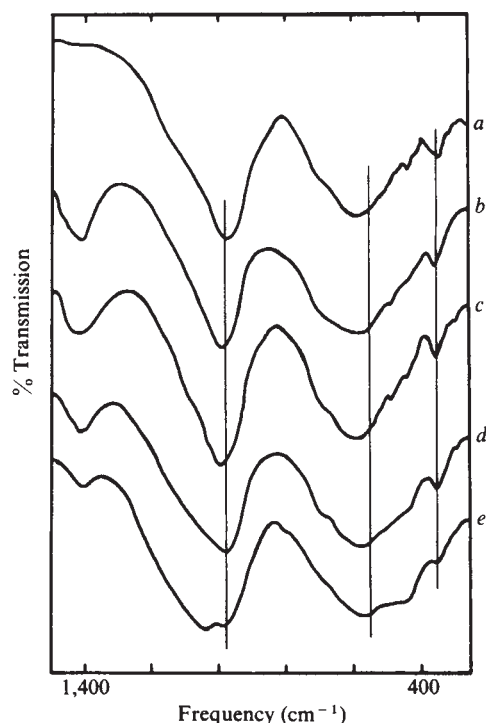


Fig. 1 Comparison of IR absorption spectra. Synthetic gel, *a*; podsol separates, *b* (fine clay) and *c* (coarse clay); and fine clay separated from Taupo sandy silt, *d* (B horizon) and *e* (C horizon).

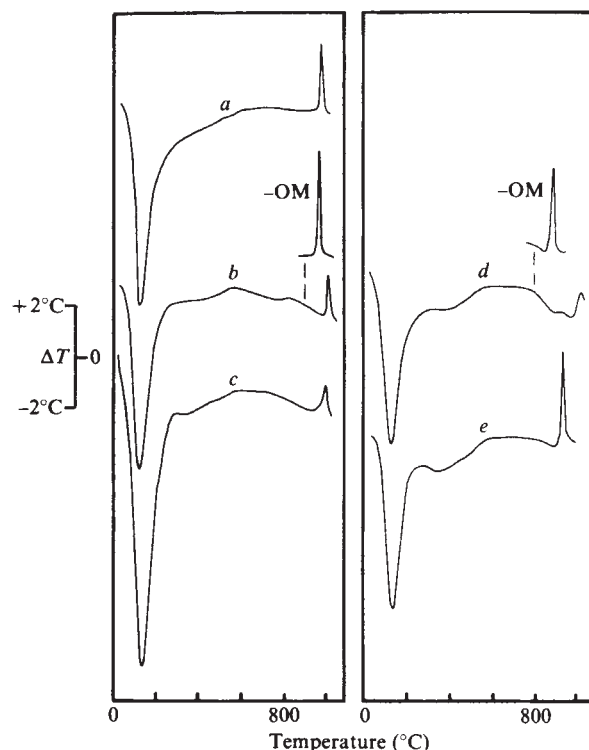


Fig. 2 Comparison of DTA thermograms. Identification of samples as for Fig. 1. Experimental conditions: sample masses, 50 mg (podsol), 80 mg (remainder); nitrogen atmosphere; -OM indicates that natural organic matter was removed by burning, 10 mg charcoal added, and sample reheated in nitrogen atmosphere.

minium-rich component. Unpublished data obtained in this laboratory have shown oxalate to be more specific than sodium hydroxide as an extractant of poorly ordered aluminosilicate gels in the appropriate experimental conditions. The $\text{SiO}_2/\text{Al}_2\text{O}_3$ mole ratios of the oxalate-extracted phases from both the podsol and Taupo samples are about half of the value of 1.0 commonly cited for allophanes¹³. Note that the reactive gels from the Taupo clays are more aluminous than those from the podsol, but hydroxyl release by fluoride⁵ indicates that the podsol contains the greater amount of allophanic material. The considerably higher $\text{SiO}_2/\text{Al}_2\text{O}_3$ mole ratios of the material extracted by sodium hydroxide from the podsol coarse clay and both Taupo clays indicates that this reagent has also partially dissolved the more siliceous phases known to be present in these samples.

The thermal characteristics of the fine and coarse clay from the podsol are almost identical to those of the Taupo allophanes and the synthetic gel (Fig. 2). All meet the defined requirements for allophane¹⁴. In addition, the effects of particle size, organic matter and carbon on the high temperature exotherm given by the podsol gels are consistent with those shown by allophane^{10,15}.

Fieldes has postulated that co-precipitation of weathering products in the subsoil of podsoils could lead to the formation of allophane¹⁶. Clearly, from the data presented here, the poorly ordered gel isolated from a podsol meets the widely accepted criteria for allophane. We have therefore confirmed that the genesis and persistence of allophane in podsoils is possible, and that its genesis does not require the presence of a poorly ordered parent material.

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Gyromagnetism and the remanence acquired by a rotating rock in an alternating field

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Although alternating field (a.f.) demagnetisation is routinely used in palaeomagnetism it has relatively recently been recognised that rotation of rock samples in the presence of an a.f. may introduce unwanted components of magnetisation. Doell and Cox¹ observed such magnetisations in 1967 but the phenomenon was not attributed to the effect of rotation until Wilson and Lomax² performed experiments about a single rotation axis and demonstrated the remarkable result that when an alternating field was applied at right angles to the axis of slow rotation, a remanent magnetisation was left antiparallel to the rotation vector after the a.f. had been smoothly reduced to zero. This magnetisation has been termed rotational remanent magnetisation (RRM). One of the explanations considered in attempts to account for the effect is that it is gyroscopic in origin, but order of magnitude calculations based on the rotation rate of the rock have indicated that such effects are insignificantly small^{2,3}, especially as RRM is detectable at rotation rates as low as 0.02 revolutions per second (r.p.s.). It is reported here that if appropriate assumptions are made regarding the magnetic behaviour of the rock, gyroscopic effects may be large enough to explain RRM.

When a rock is rotated at an angular frequency ω in a zero field, any magnetic moments within it will incline towards the rotation axis and a magnetic moment will appear antiparallel to the rotation vector. This is known as the Barnett effect and is a consequence of the intrinsic gyroscopic properties of the uncompensated electron spins which give rise to the moment of each magnetic particle. If the rock is static, the equivalent axial field B required to produce the same inclination of the moments towards the rotation axis is given by $\omega m/e$ where e/m is the ratio of charge to mass for the electron and where the magnetic properties of the particles within the rock are assumed to be due to electron spin rather than orbital motion. This equivalent field acts antiparallel to the rotation vector and is of magnitude $f_r \times 3.6 \times 10^{-11}$ T where f_r is the rotation frequency in revolutions per second (r.p.s.). For rotation rates of even several hundred r.p.s. it is thus insignificant compared to the fields required to produce a measurable remanence.

When, however, the rock sample is rotated in an alternating field, the changes in magnetisation which the rock experiences may be illustrated by Fig. 1, where for simplicity of explanation the alternating field has been represented by a series of moderately sharp pulses of alternate sign. Instead of considering

an anticlockwise rock rotation, Fig. 1 has been drawn with the rock stationary and the field axis rotating clockwise around it. A simplified explanation of the magnetisation changes in Fig. 1b are as follows. During the first pulse an isothermal remanent magnetisation (IRM) is acquired by the rock in position 1. Because the field axis is slowly rotating, the second pulse arrives at position 2 and as it builds up to its maximum value, it gradually removes the IRM acquired in position 1, modifying it by first flipping the low coercivity part anticlockwise towards the new direction but still exerting a torque on the high coercivity part which remains. This too is finally removed when the field reaches its maximum. During this build up of the second pulse, a positive (accelerating) torque (that is, in the same sense as the actual rock rotation) acts on the rock in Fig. 1b, c and d which are drawn for the rotation frequency (ω) less than the a.f. frequency (Ω). In Fig. 1e, however, where ω exceeds Ω , the torque is negative (decelerating) and the flip of the magnetisation is clockwise. This process is repeated in succeeding cycles.

During each flip, the rotation rate of the magnetic moments within the rock might be expected to be of the order of the reciprocal of the switching time or time constant (τ_f) of the flip. This may well depend on the coercivity of the particle but might be expected from a consideration of spin dynamics⁴ to be of the order of less than 10^{-8} – 10^{-7} s that is, the rotation rate of the magnetic moments during the flip will be of the order of 3×10^7 r.p.s. or greater. If the rock is stationary, as many moments will flip clockwise as anticlockwise and the system has perfect symmetry. Once rotation occurs, however, an asymmetry in the numbers of moments flipping in a particular sense is introduced and a corresponding torque will also be present as described above. The effective axial field B_g , which can be considered to deflect each moment towards the rotation axis as it flips towards the field direction, will thus be at least some 3×10^7 times greater than the value of 3.6×10^{-11} T quoted above, that is, about 1 mT or greater. This effective field is not a real field any more than is the anisotropy field, for example, but like the latter it is a useful concept. It is of the order of $\pi m/e\tau_f$ and will act in a direction antiparallel to the rotation vector describing the flip. For $\omega < \Omega$

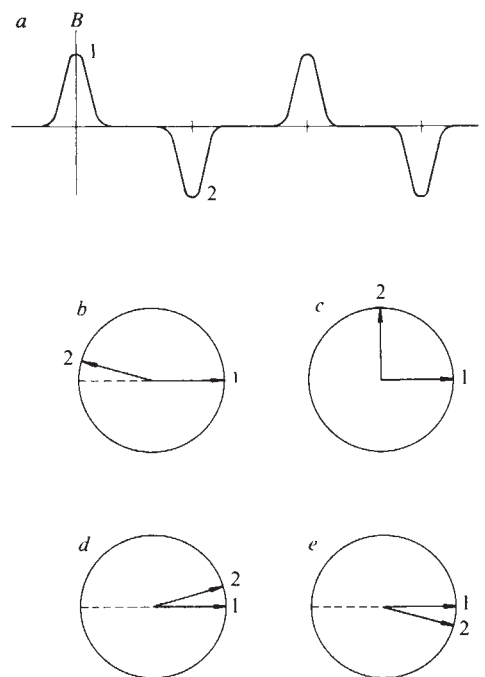


Fig. 1 a, a.f., for simplicity represented by a series of pulses. b, Direction of the first two field pulses as the field axis rotates clockwise around the cylindrical rock sample at a low rotation frequency (ω): c, when ω is half the a.f. frequency (Ω); d, when ω is just less than Ω ; e, when ω is just greater than Ω .

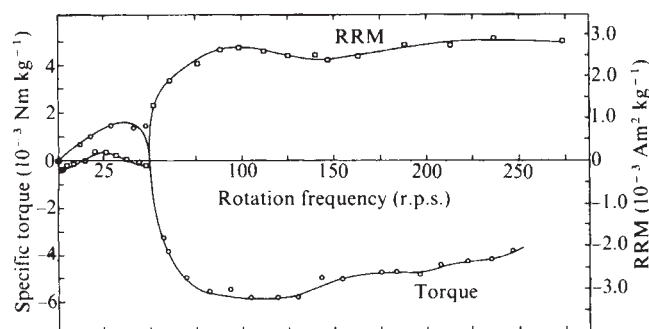


Fig. 2 The dependence of torque and RRM on rotation frequency for a peak a.f. of 51 mT for igneous sample WD34.

the flip is anticlockwise in Fig. 1, that is, in the same sense as the actual rotation of the rock, and thus the effective field is antiparallel to the rotation vector (that is, negative). When $\omega > \Omega$, however, the flip is clockwise and the effective field is thus positive. A transient effective axial field of the order of 1 mT acting just when the particle moments are flipping might be expected to produce a similar axial magnetisation to a constant field of 1 mT acting in the presence of the same transverse a.f. with no rock rotation if the sense of the flip were the same for all moments.

This discussion is clearly greatly simplified as the motion of each moment during the irreversible flip will presumably be that of damped precession⁵ under the influence of the external field and the anisotropy field, the latter reversing sign when the moment crosses the energy barrier separating the two equilibrium positions (for uniaxial anisotropy). To avoid an analysis of this complex motion the approach used above is an attempt to simplify the problem by considering the overall bias which is likely to be imparted to the motion of the particle moments (as a result of gyroscopic effects) as they are forced to flip by the external field. This bias is then interpreted as being due to an equivalent field B_e and the remanence which results from the action of this equivalent field is considered to be similar to an anhysteretic remanent magnetisation (ARM) acquired in a constant field in the presence of the much larger peak a.f. RRM is thus interpreted (like ARM) as being due to a statistical partial alignment of moments caused by a biasing field (B_e) in the presence of a much larger a.f. Because the moments do not all flip in the same sense, however, (the role of rotation is to introduce a bias favouring one particular sense of flip), the maximum RRM observed might be expected to be rather less than the ARM acquired in 1 mT in the conditions described above. The sign of RRM from this analysis is in agreement with the results of Stephenson³ who investigated RRM up to rotation speeds of 60 r.p.s.

To investigate RRM at higher rotation speeds, measurements were made on two igneous samples which were rotated at frequencies between 1.5 and 275 r.p.s. in a peak alternating field of 51 mT (ref. 5). This range of speeds was achieved by using a turbine driven from a compressed-air supply, with the rock sample mounted in the rotor. The magnetic torque exerted by the alternating field was measured by observing the initial rate of change of angular momentum imparted to the sample plus rotor when the field was suddenly applied to the stably spinning rotor⁶. The RRM was given to the sample by spinning the sample at the desired speed, applying the a.f. by quickly turning up the current in a surrounding coil by means of a Variac, and then after a second or two, smoothly reducing the current to zero over a few seconds.

Figure 2 shows the RRM and torque results for one of the samples coded WD34. In accordance with the above explanation, RRM is negative (except between 12 and 40 r.p.s.) for $\omega < \Omega$, and positive for $\omega > \Omega$. The fact that neither RRM nor torque exhibit further sign changes at higher rotation rates,

which might be expected from the simple explanation of Fig. 1, is because the a.f. is not, in fact, a series of pulses and the IRM acquired during the decreasing part of the cycle is 'smeared out' over a range of angles so that the torque is then always negative (retarding). A two dimensional numerical model of the rock based on single domain particles and a coercivity spectrum determined from the acquisition of IRM as a function of applied field has enabled a torque curve to be computed which is very similar to the above experimental result⁵.

The above description is greatly simplified, and, while the numerical model is more sophisticated than this, it is by no means a complete magnetic description of the rock, and therefore the unexplained experimental observation of the secondary sign change between 12 and 40 r.p.s. is possibly due to deficiencies in the model rather than because the above explanation is necessarily at fault. This secondary sign change was not so marked in the other sample and did not occur in the two samples investigated by Stephenson³.

The direct field required to produce an ARM of the same magnitude as the maximum RRM observed was about 100 and 20 μ T for the two samples (in a peak field of 136 mT rather than 51 mT). The estimated 1 mT transient effective field, which is thus a consequence of the sudden flip of the moments, which occurs when the field strength and direction becomes favourable would thus seem to be easily strong enough to account for the phenomenon of RRM. Moreover, this mechanism does not depend on the absolute rotation rate of the rock but only on the rotation of the field relative to the rock. This is in agreement with experimental results of Stephenson who found that it is immaterial whether the rock rotates or whether the a.f. axis rotates about the stationary sample³.

Perhaps RRM may thus be an example of a new type of remanence for which the name 'gyroremanent magnetisation' or GRM might seem to be appropriate.

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A gyroremanent magnetisation in anisotropic magnetic material

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An effect whereby an anisotropic magnetic material acquires a remanence on exposure to an alternating magnetic field (a.f.) is predicted from an analysis of the phenomenon of rotational remanent magnetisation (RRM). The remanence, which is of gyromagnetic origin, is produced even when there is no relative rotation between sample and field and all constant fields have been eliminated. The effect is demonstrated in magnetic recording tape and, as well as having important implications for a.f. demagnetisation techniques, may be relevant to magnetic recording processes.

The phenomenon of gyromagnetism is well known (see ref. 1 for a review) but it has not generally been considered that it could ever produce an easily measurable remanent magnetisation. It has recently been suggested, however^{2,3}, that the RRM identified by Wilson and Lomax in rock samples⁴, can be explained in terms of a gyromagnetic effect associated with a predominant sense of flip of the moments inside the sample as it

is rotated in an alternating field. In a simplified approach to the complex problem of determining the motion of the particle moments during the flips, it has been suggested that, because of the sudden enforced rapid rotation of their associated angular momentum vectors, the particle moments can be considered to be deflected towards the rotation axis by a transient field B_g of the order of $\pi m_e / e \tau_f$ (where e/m_e is the charge to mass ratio for the electron and τ_f is the time of flip of the moment). It is this effective transient axial field, acting during the flip, which produces the remanence, and therefore in any system where a particular sense of flip predominates, such a magnetisation should result. Thus a rotating field of constant amplitude should also produce a remanence antiparallel to the field rotation vector, provided that the field is strong enough to produce the irreversible flips of the moments. In such a case, the resultant magnetisation should be independent of the rotation frequency f , provided that $1/f \gg \tau_f$. Of even greater interest, however, is that this approach leads to the prediction that rotation, either of the magnetic material or the magnetic field, is not a necessary condition for gyroremanent effects to produce a remanence. This remanence, here termed a gyroremanent magnetisation (GRM), is likely to be produced whenever there is an asymmetry in the number of moments which flip in a particular sense.

Consider a uniaxial single domain particle orientated at an angle θ to an alternating field (see Fig. 1). Successive pulses of a weak a.f. merely cause reversible excursions of the moment away from the easy axis but a stronger field will cause the moment m to flip between the two easy directions (actually between two positions which are governed by the anisotropy field and θ). If θ is positive as in Fig. 1a, the sense of the flip will always be clockwise. That is, the rotation vector describing the flip is in the negative z direction. If, however, θ is negative as in

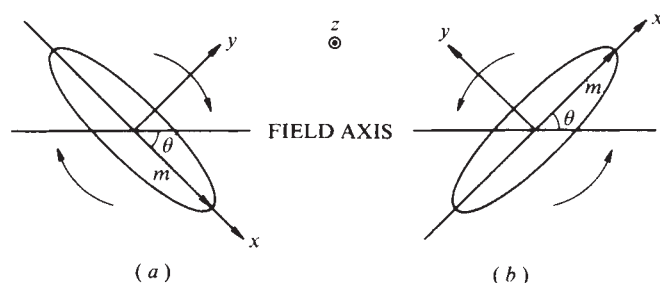


Fig. 1 A diagram to show the sense of the irreversible flip of the moment of a single domain uniaxial particle when subjected to an alternating field with peak value comparable to the anisotropy field.

Fig. 1b, the flip will be anticlockwise. As a result of the flip and because of the intrinsic gyroscopic behaviour of the moment of the particle, the moment will be deflected towards the $+z$ direction in Fig. 1a ($-z$ in Fig. 1b)^{2,3}. Clearly in the case under consideration, no remanence can be retained antiparallel to the flip vector because the moment will always lie along one of the two easy directions when the field is removed. In an assembly of particles, however, such as in a rock, where each particle has three unequal orthogonal axes representing its anisotropy, and where there are many different particle orientations, any inherent bias imparted to the overall motion of the moments of the assembly during the flips would be expected to produce statistically a remanent magnetisation in the opposite direction to the average flip vector in an exactly analogous way to that discussed previously in connection with RRM^{2,3}. In an anisotropic rock, therefore, with its easy magnetisation axis orientated along the x axis in Fig. 1 (making use of the same diagram), the bulk anisotropy will cause the isothermal remanence acquired in each pulse of the a.f. to be deflected towards the nearest easy direction, so that the same sense of flip as illustrated will predominate and an effective field antiparallel to the average flip vector will be present.

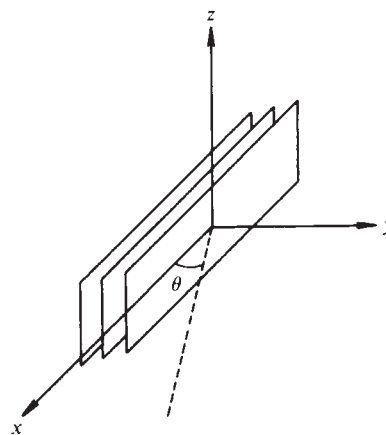


Fig. 2 Orientation of 100 layers of magnetic recording tape. The dashed line in the x - y plan indicates the a.f. axis for positive θ as in Fig. 1a.

This effective field will thus produce a GRM perpendicular to the a.f. and anisotropy axes. The direction (but not the magnitude) of the GRM will be given by the vector $\mathbf{A} \wedge \mathbf{F}$ where $\mathbf{A}$ is a vector along the anisotropy axis. The field axis is specified by a vector $\mathbf{F}$, the direction of which is such that θ (the angle between $\mathbf{A}$ and $\mathbf{F}$) always lies between $-\pi/2$ and $+\pi/2$. As the orientation of the field axis is thus altered through $\theta = 0$, the sign of the GRM should thus change, being negative (along the $-z$ direction) for $\theta < 0$, and positive for $\theta > 0$. The magnitude of the GRM might be expected to depend on θ , the flip time (τ_f) of the particle moments, and the magnitude of the anisotropy. For a very anisotropic sample, GRM might be expected to be of similar magnitude to the ARM acquired in a transverse a.f. of the same strength as that used to produce the GRM, and in the presence of a steady field of magnitude $\pi m_e / e \tau_f$.

To test this hypothesis an anisotropic sample was fabricated by stacking together 100 strips of magnetic recording tape (see Fig. 2). Each strip was of dimensions 18 mm $\times$ 6 mm. The long axis of each strip (x axis) was mounted horizontally in a non-magnetic holder with the plane of each strip vertical (z axis).

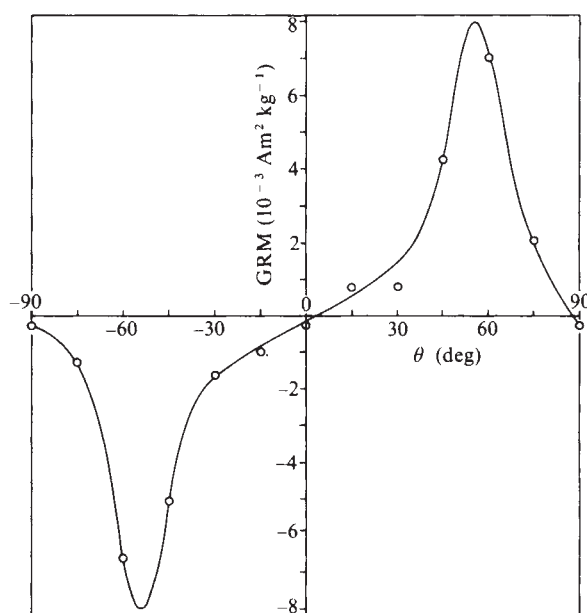


Fig. 3 The dependence of the z axis magnetisation (which is a GRM) for 100 layers of magnetic tape when subjected to a 274 Hz alternating field of 50 mT peak at an angle θ as in Fig. 2.

Initial susceptibility measurements yielded values of 128 and $85 \times 10^{-6} \text{ m}^3 \text{ kg}^{-1}$ respectively (per unit mass of tape), in directions along and perpendicular to the plane of the strips. Because the distribution of the particles is essentially isotropic in the x - y plane⁵ these differences in susceptibility are due to the differences in demagnetisation factors in the two directions. Nevertheless this shape anisotropy should produce the same sense of flip as illustrated in Fig. 1 when the x direction coincides with the long axis of the tape.

The tape was demagnetised by tumbling the composite sample in an a.f. demagnetiser which reduced the remanence to a suitably low value. This was measured on a spinner fluxgate magnetometer used for measuring the remanence of rock samples. The sample was then orientated at various angles as in Fig. 2 inside a set of Helmholtz coils which cancelled the Earth's field. The a.f. was increased to a peak value of 50 mT (at 274 Hz) and then slowly decreased over a time of about 1 min and any resulting remanence was measured. Between each different orientation the sample was demagnetised by tumbling. A z -axis remanence did indeed result from this procedure and this is plotted as a function of θ in Fig. 3, where it is apparent that the GRM has the general form expected from the preceding analysis. This result should be independent of a.f. frequency f , provided that $1/f \gg \tau_r$.

When the pair of Helmholtz coils cancelling the Earth's vertical component was switched off and a field of $44 \mu\text{T}$ acted along the $-z$ direction, the magnetisation changed from 7 to $-21 \times 10^{-3} \text{ Am}^2 \text{ kg}^{-1}$ at $\theta = +60^\circ$. This suggests that an ARM of about $28 \times 10^{-3} \text{ Am}^2 \text{ kg}^{-1}$ is being added in opposition to the

GRM, so that the effective field producing the GRM, at least when the peak a.f. is 50 mT, is equivalent to a steady ARM field of about $11 \mu\text{T}$. This value is not unlike those estimated from RRM experiments on rocks^{2,3}.

The implications of this result are considerable for the a.f. demagnetisation of anisotropic rocks, since it implies that a single axis non-tumbling demagnetisation system will invariably introduce a GRM into the sample unless the anisotropy axes of each rock happen to be orientated at 0 or 90° to the a.f. axis. Such magnetisations in the past may well have been misinterpreted as ARM, but an orientation dependence check should indicate the presence of a GRM. Such a dependence is now under investigation in a rock sample and will be described at a later date. This effect may also turn out to be of importance in magnetic recording. It may also prove to be a useful tool in studying magnetisation rotation process in magnetic materials and anisotropy in rocks and minerals.

Of perhaps even greater interest, however, is that gyromagnetism can produce an easily observed remanence in magnetic materials even when they are merely exposed to an alternating field, and this would appear to be an effect which has hitherto been unsuspected.

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A braidplain facies model for the Westphalian B Coal Measures of north-east England

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The Westphalian B (Coal Measure) sediments of Northumberland, Durham, and the other northern English coalfields were deposited on extensive coastal plains between several peripheral source areas and the Saint George's Land block to the south. We report here a facies model resulting from studies of fluvial sandstones in the Westphalian B of Northumberland and Durham and suggest that sandy braidplains were an important feature of the depositional environment in this coalfield. Braidplain sand sheets, and associated washouts in underlying coal seams, formed following tectonic uplift of local source areas supplying recycled sands. Parts of this model may be extended to other coalfields in northern Britain.

It has often been assumed that the Coal Measures environment was a large-scale delta top^{1,2}, with peat-forming swamps laterally equivalent to meandering rivers, and a marine influence becoming more important towards the central Pennine coalfields³. Detailed interpretations of parts of the Pennine coalfields have been made as the deposits of swamp, lake, lake delta and river channel environments^{4–6}, but no model to explain the major sedimentary features of this coalfield, or the differences between the different Pennine coalfields, has emerged.

In the Northumberland and Durham coalfield the Coal Measures can be divided into three main sedimentary facies associations. (1) The marine band facies consists of distinctive fossiliferous silty mudstones up to 2 m thick. These are interpreted as the deposits of occasional, low energy marine transgressions. (2) The coal-bearing facies consists of part or all of a sequence which, when complete, comprises black fossiliferous

shales, passing up into grey, finely laminated mudstones and siltstones and overlain by seat-earth and coal. These siltstones may also contain very fine-grained sandstones, forming sheets 10–50 cm thick, of limited lateral extent, and often containing bioturbation, climbing ripple or trough cross-lamination structures. This is the dominant facies association; it ranges from 10 cm to 15 m in thickness and is interpreted as the deposits of shallow lakes with small delta infills, capped by swamp vegetation. (3) The major fluvial facies comprises fine to very coarse sandstones 4–35 m thick, with no autochthonous coal. Each sandstone has an erosional base and fills any washouts in the underlying coal seam, so any understanding of these major sandstones and their associated washouts is of great economic importance to coal exploitation. The lower part of the sandstone is often conglomeratic, containing locally eroded clasts of ironstones and angular partially lithified silts, together with clasts of coalified once-leathery peats and fossilised log jams. The sequence passes up into trough and tabular cross-bedded sandstone in sets 1–3 m thick forming cosets 5–15 m thick with unimodal palaeocurrent vectors paralleling washout trends. These are followed by about 2 m of ripple-laminated siltstones and fine sandstones, and may be capped by seat-earth and coal of the coal facies. There is no evidence for any significant systematic lateral river migration. This type of sequence is interpreted as the deposits of a river in which a rapid, unidirectional flow decelerated, allowing silting of the channel and eventual blanketing by vegetation. Field and borehole data show that these sandstones form thin sheets, usually 10–15 m thick, but occasionally up to 35 m in thickness. These can cover large areas of the coalfield ($>1,500 \text{ km}^2$), but are not continuous, leaving occasional 'islands' of older sediments. These major stratigraphic and facies relations are quite unlike those of migrating and avulsing fluvial systems⁷ and we suggest that the vertical sequences are not due to the migration of laterally equivalent facies, but rather to major environmental changes with time. These sandstone sheets probably resulted from the periodic invasion of high-energy braided rivers eroding into and spreading over an otherwise low-energy coastal plain, to form large areas of sandy braidplain (Fig. 1). There were no major deltas. This implies periodic rejuvenation of the river system by changes in discharge or of slope, possibly originating from

tectonic, climatic or eustatic changes. Thus in any one seam, washouts were formed synchronously after river rejuvenation when the stream power was rapidly increased, but before erosion produced a significant sediment load. Consequently, many washouts are narrow, deep and of low sinuosity, but as the rivers lost power and aggraded vertically, they continued to widen, often by eroding along the tops of peat seams which formed underlying cohesive layers (Fig. 2). Because these major sandstone events were time horizons, they may be of value for seam correlation.

In Northumberland and Durham several braidplain sandstones fill washouts trending east/west, whereas only a few fill north/south washout trends. Palaeocurrents deduced from major bedforms and coincident washout trends may be directed eastwards or northeastwards in one sandstone, and be succeeded by westward-directed palaeocurrents and washouts in the next. This periodic palaeoslope reversal implies tectonic tilting from sandstone to sandstone. The palaeoslope reversal is also reflected in a difference in lithology between successive sandstones, suggesting different source areas. For example, a sub-rounded/rounded subarkose with orthoclase and minor muscovite may be succeeded by a sub-rounded subarkose with perthitic and microcline feldspars, and minor garnet. All of these features can best be explained by repeated tectonic uplift and erosion of local sedimentary source areas to the west, north and east, rather than by a single large river system depositing far-travelled detritus. Note that this province was probably tectonically active during the Westphalian^{13,14}. Thus it is possible that much of this mature, rounded, sandy detritus was reworked from easily eroded areas of Namurian or older sediments on the Southern Uplands, Mid North Sea High and North Pennines. Such source areas only a few tens of kilometres distant need only have been uplifted by a few tens of metres to have triggered off erosion and provided a slope sufficient to maintain braiding in sandy sediment, given a reasonable discharge variation^{10,15,16}. Away from the source areas river slope decreased. This caused a decrease in river power, deposition of the coarser

bedload, and a transition to meandering rivers further southwards (Fig. 1). After the source areas had been reduced by gullying, removing any stored sediment¹⁰, the braidplain would silt up, and the coal facies re-establish. Occasionally, some of the large braidplain events influenced the distal central Pennine areas, causing unusually widespread washouts and sands. The silts and very fine sands normally deposited on this coastal plain show evidence of river migrations and avulsions^{4,5}, however, these features were only well developed in the distal parts of the overall coastal plain.

Climatic change is unlikely as a cause of this river rejuvenation, as such a change would have had to have been repeated rapidly many times within the Westphalian B and would not explain such divergent palaeocurrents and source areas.

Worldwide eustatic sea-level changes are not favoured as the cause of river rejuvenation because of the palaeocurrent, petrographic and proximal to distal features mentioned above. During eustatically induced regressions^{8,9}, any fluvial transportation would be expected to reach across former shelf or coastal plain areas, and deposit sands in shoreline areas^{8,10,11}. While each regression continued, erosive gaps and a recurrent linear washout trend down the palaeoslope would be expected to have developed in these coastal shelf areas. However, the evidence above, together with borehole information from Northumberland and Durham, does not support this in the Westphalian B.

The idea that the Northumberland, Durham and probably Cumbrian coalfields were slightly elevated proximal areas with respect to the other Pennine coalfields is supported by regional marine band variations. These proximal coalfields have thinner marine bands containing a low diversity near-shoreline fauna and several bands were not deposited at all³. This suggests that the sea was unable to transgress into these proximal areas as often, or for as long a period, as elsewhere. The Mid-Westphalian A to Mid-B has very few marine bands and contains the most productive coal seams¹⁷. The prevailing regional subsidence may have been matched by clastic supply¹² during this

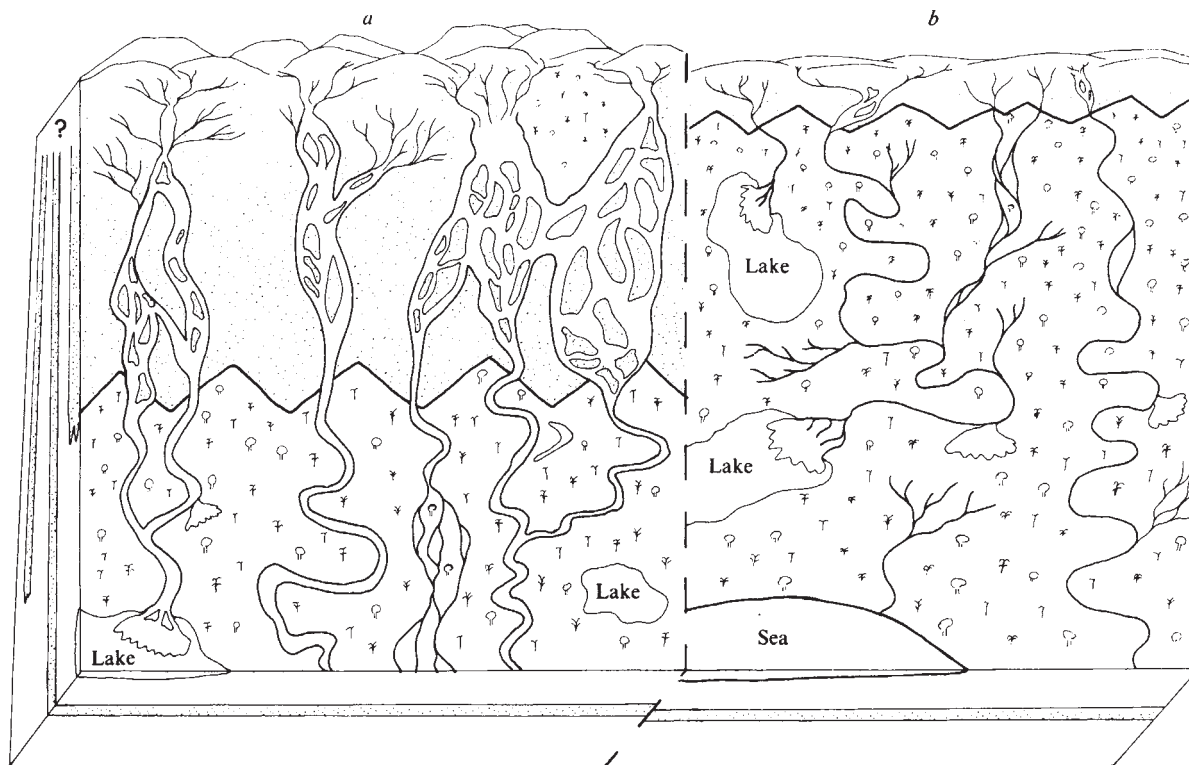


Fig. 1 Schematic palaeogeographical block diagram showing large-scale environmental changes in Coal Measures. Not drawn to scale, but a distance of several hundreds of kilometres from source areas to sea is envisaged. Plan shows proximal to distal changes proposed for: *a*, a period of maximum braidplain development, passing into coastal plain swamp. Source areas were uplifted. *b*, The coastal plain swamp encroached as the braidplain was abandoned. Source areas now eroded. Block section shows the extent of previous braidplains, but not detail of coal or marine facies.

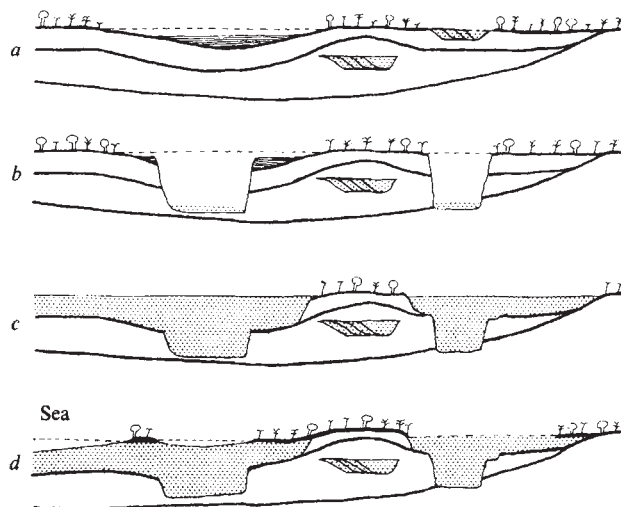


Fig. 2 A schematic cross-section perpendicular to palaeocurrent flow shows the sequence of events resulting from river rejuvenation. Horizontal scale greatly shortened. *a*, Coastal plain coal facies, fed by small low energy avulsing and meandering rivers, which deposited predominantly fine-grained sediments and formed minor lake deltas within a swamp environment. *b*, Uplift of source area by only 10–20 m, not necessarily synchronous or of equal amount in all source areas, caused river rejuvenation. Rivers formed new channels along lines of least resistance through topographically low areas such as pre-existing rivers, or old lake basins. These rivers initially carried little coarse sediment and rapidly cut down into the older coastal plain sediments, forming low sinuosity washouts where peat was eroded. *c*, In areas proximal to source, the rivers quickly received coarse sediments and aggraded. They then rapidly widened along the top of the cohesive peat layers by bank collapse, forming basal conglomerates. Sand load continued to be deposited in proximal areas forming a braidplain, and usually only the fine sediments were carried down to distal coalfield areas, where washouts from the initial increased water discharge were filled with silts and fine sands. *d*, As the source area became rapidly eroded and aggradation continued, a near equilibrium base level was restored and rivers lost their power and silted up to form large shallow lakes. The distal coastal plain coal facies gradually encroached northwards and swamp conditions resumed with steady state graded rivers. If regional subsidence was too rapid, the sea transgressed, forming a marine band.

period. Conversely, in the Upper B, regional subsidence often outpaced supply, resulting in many marine transgressions. It seems likely that such transgressions had a significant tectonic and sedimentary control independent of eustatic sea-level changes. The large time range estimated for a goniatite zone fauna⁹ may be significant in the correlation of some apparently more widespread marine bands.

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Silurian or Upper Ordovician fossils at Guolasjav'ri Troms, Norway

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In August 1979, specimens of rugose and tabulate corals, crinoids and brachiopods (Fig. 1*a*) were recovered from meta-limestones in a belt extending 0–3.5 km north-west of Guolasjav'ri, a mountain lake south-east of Kåfjord, Troms, in Norway (Fig. 2, locality I). This is the first fauna to be recognised in this part of the Caledonian nappe sequence in Troms. The presence of corals proves that these metalimestones are not older than Middle Ordovician which imposes constraints on the correlation of this series with units in the Caledonides to the north and south and has an important bearing on the problematic relationship between the dominant Scandian phase (mid-Silurian¹) of the Central Scandinavian Caledonides and the Finnmarkian phase (late Cambrian²) of northernmost Scandinavia.

Padgett³ erected the first lithostratigraphy in this part of the Norwegian Caledonides (Fig. 3). Within his 2,600 m non-granitised Birtavarre Series, he named the unit in which the fauna has been found the Guolas Limestone Series. Where the fossils are preserved, this has undergone Barrovian-type, amphibolite-facies regional metamorphism synchronous with polyphased deformation (our unpublished data). These fossils are therefore poorly preserved, although they remain surprisingly little deformed. The two least altered specimens are identified as halysitids with ordinary lacunae and elliptical corallites about

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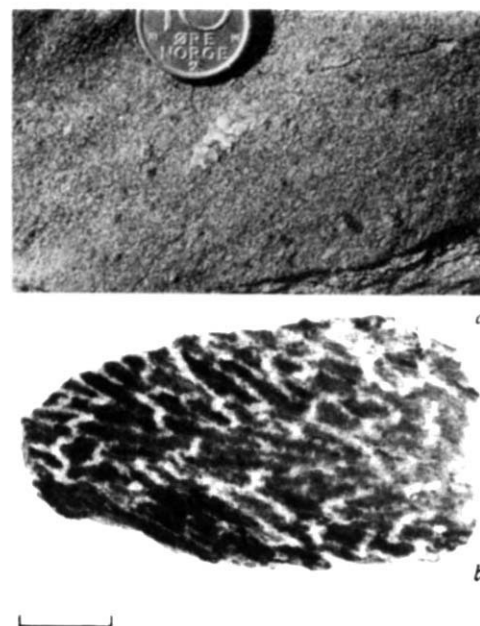


Fig. 1 Photographs of the fossils. *a*, Crinoid ossicles and a fragment of a ribbed brachiopod at outcrop locality 1 (Fig. 2). Coin is 1.5 cm in diameter. *b*, Halysitid tabulate coral, cut surface; from locality 2 (Fig. 2). Scale bar, 0.5 cm.

1 mm wide in cross-section (Fig. 1b), indicating a Silurian or possibly late Ordovician age.

Although tectonic dislocations (for example, the Cappis Thrust) were recognised³ within the Kåfjord sequence, the stratigraphy was not thought to have been disrupted significantly. Another thrust^{4,5}, however, below the lower quartzite and marble units, not recognised by Padgett, forms an important regional nappe boundary (see for example, ref. 6 and Fig. 3). Recent field work (largely by R.E.B. to be detailed elsewhere), also supported by evidence farther afield, has demonstrated a simpler stratigraphy than that of Padgett and other workers^{7,8}. The succession (Fig. 3), including horizons of intermediate or acid lavas in a 350–400 m thick basic pillow-lava accumulation near locality I, appears to be repeated by large-scale, sub-recumbent folds. Way-up evidence (mainly from pillow shape and the clast content of an immediately overlying polymict conglomerate) supports a normal stratigraphic order in the fossiliferous part of the sequence just north of Guolasjav'ri.

One of the outstanding problems of the Kåfjord sequence has been its correlation within the Scandinavian Caledonides. Zwaan and Roberts⁸, although recognising repetitions within Padgett's³ Kåfjord stratigraphy, which they sought to explain entirely in terms of thrusting, correlated these rocks, in accord with the orthodox view^{2,9–11}, with the upper portion of the well documented Sørøy stratigraphy¹² (Fig. 2). The late Precambrian–late Cambrian age of the latter is based on an early to middle Cambrian archaeocyathid fauna¹³ and on radiometric age dating (see refs 2, 14). Quenardel's⁷ interpretation seems to be largely a combination of those of Padgett³ and Zwaan and Roberts⁸. In contrast, some economic geologists¹⁵ postulated an Ordovician age for the ore-bearing rocks within the Kåfjord sequence on account of the similarity in ore paragenesis between these sulphide ores and some farther south in Scandinavia.

The lithostratigraphic correlation between the Kåfjord and Sørøy sequences led Sturt, Roberts and others^{2,7,8,11,16} to assume without reservation that the late Cambrian Finnmarkian orogenic phase extended through the Kåfjord area and much farther south into Nordland and adjacent parts of Sweden. They thus discounted the previous correlations^{17–20} between the southern part of the area and fossiliferous Ordovician–Silurian (Köli) rocks a little to the south. The discovery of a late Ordovi-

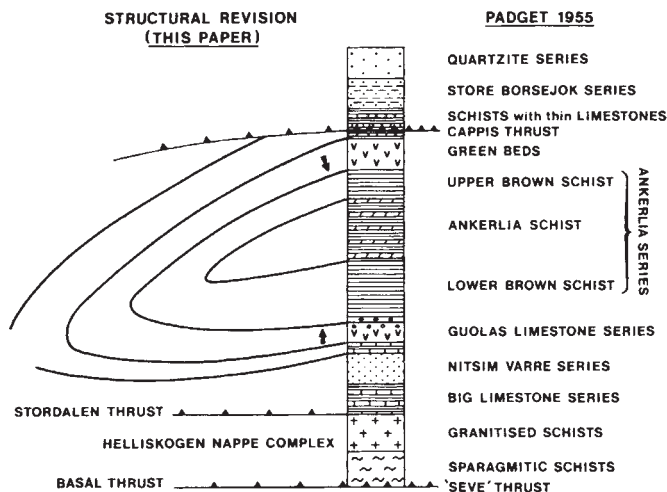


Fig. 3 The interpretation of the stratigraphy of the Kåfjord region according to Padgett³ (right) and the revision proposed in this work (left). The column is not to scale—the sequence above the Stordalen Thrust (Padgett's non-granitised Birtavarre Series) is ~2,600 m thick.

cian–Silurian fauna in the Kåfjord rocks which are shown to belong to a stratigraphic sequence only disturbed by tectonic duplications, makes the lithostratigraphic and tectonic correlations of this sequence with that of the Köli and Gasak nappes^{18,20} of central Nordland and adjacent parts of Sweden discussed by Binns⁶ more likely. Geochemical comparisons between the volcanic rocks of the Kåfjord and Köli sequences are under way and these may strengthen this correlation. Padgett's Cappis Thrust may define the nappe boundary but lithological and tectonic complications south-west of Kåfjord prevent this from being more than a tentative proposal at this stage.

Even an approximate chronostratigraphic correlation between the Kåfjord and Sørøy sequences can no longer be entertained. It seems possible to trace the Kåfjord sequence laterally through to the Kvaenangen–Øksfjord area^{3,4,8,9,23,24} (Fig. 2). At Øksfjord the strata are invaded by the syn-orogenic (Finnmarkian phase; climax 500–550 Myr ago²) Seiland Igneous Complex^{21,22}. If the lithostratigraphic correlation and the Sørøy region isotopic age evidence are to be relied on, it is difficult to explain the ensuing relationship without invoking rapid diachronous southward younging of the active orogenic belt and of regional sedimentation during late Precambrian–late Silurian time. Neither geophysical work²⁵, nor mapping^{9,21,24} have revealed a major transverse tectonic break between Øksfjord and Kvaenangen and tectonic interleaving of older and younger nappe units is incompatible with the correlation of Hooper and Gronow²³ and Armitage *et al.*⁹. Equally rapid tectono/thermal diachroneity has been recognised in Cenozoic triple plate junction migration²⁶ but sedimentation diachroneity of at least 100 Myr in a lateral distance of about 100–200 km (an estimate of pre-folding relationships) seems inconceivable. Hence we conclude that the correlations between Øksfjord and Kåfjord, and the Sørøy region isotopic age evidence require critical re-consideration. Perhaps the existence of an independent Finnmarkian Orogenic belt is in doubt.

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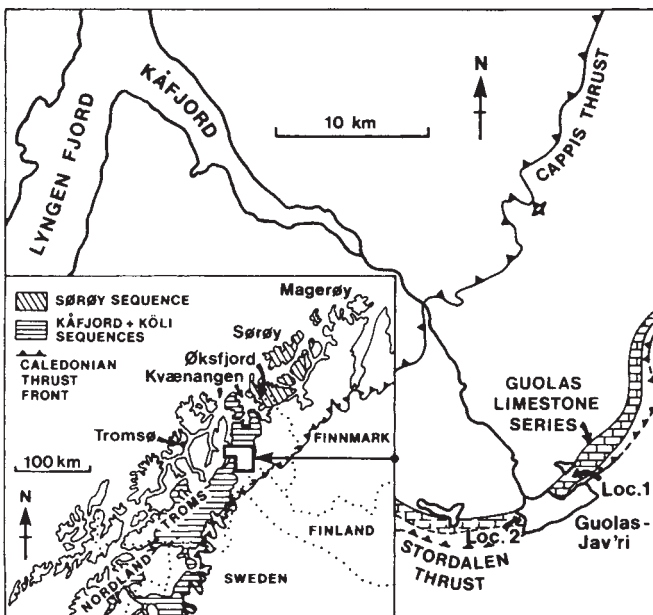


Fig. 2 Map of the Kåfjord region to show the fossiliferous localities in the outcrop of the Guolas Limestone Series. Inset shows location of the Kåfjord region within the outcrop of the Köli/Kåfjord/Sørøy sequences.

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A new hominid fossil skull (L.H. 18) from the Ngaloba Beds, Laetoli, northern Tanzania

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In 1976, a fossil hominid skull was recovered from the Ngaloba Beds at Laetoli, Northern Tanzania; its morphology is discussed here. The discovery of this skull is of great interest and importance because of its very substantial presumed antiquity and its largely anatomically modern morphology. The discovery has considerable implications for the antiquity and origin of modern *Homo sapiens*, a subject of longstanding interest and one which has gained renewed attention recently.

The Ngaloba Beds¹, lying above the vogesite lavas which separate them from the underlying Ndolanya and Laetoli Beds^{2–4}, are stream deposits, principally sandstones and claystones, of which only patches are preserved. These patches consist chiefly of detritus eroded from the underlying Ndolanya and Laetoli Beds and they contain artefacts of Middle Stone Age affinity. The skull was recovered from a 2-m thickness of the Ngaloba Beds at Locality 2 that also yielded artefacts, some fossil reptilian and avian bones as well as fossil mammalian bones. This exposure is principally of sandy claystone and contains a water-worked vitric tuff. The tuff is trachytic and contains the pyroclastic minerals biotite and anorthoclase; it is tentatively correlated with the marker tuff in the lower unit of the Ndutu Beds at Olduvai Gorge⁵. This is the only trachytic tuff younger than Bed IV in Olduvai Gorge and its age is estimated at $120,000 \pm 30,000$ yr BP (R. L. Hay, personal communication). The skull was found by E. Kandini *in situ* but eroding out of the deposits.

The skull is almost complete and includes the bones of the vault, much of the base, both temporal bones, part of the

sphenoid and much of the face including the palate and part of the upper dentition. The bones are all heavily mineralised with no signs of pathology, but there are signs of post-mortem plastic deformation that has resulted in torsion to the right of the supraorbital region and some springing of the temporo-occipital suture on the left. As recovered, the skull was in 22 pieces and coated with greyish calcareous matrix. Cleaning produced fossil bone of an ivory colour and natural texture with the preservation of remarkable surface detail. It was possible to reassemble the vault and base into one structure and the paired maxillae into

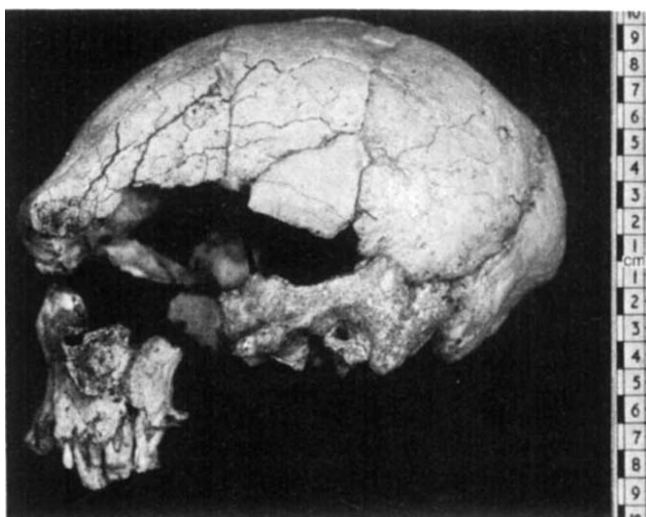


Fig. 1 Left lateral view.

another. There is no point of contact between the facial skeleton and the calvaria although very little bone is missing. The relationship between these two main fragments of the skull remains speculative. The age at death seems to have been between 18 and 30 because the sutures of the vault are all open, but one third molar is present and fully in wear. The state of wear of this tooth suggests that the upper end of the age range is most likely.

Dimensions of the skull are given in Table 1. In lateral view (Fig. 1) the skull shows several striking features including marked recession of the forehead, a rounded occipital profile, an undercut central occipital torus and a small mastoid process. The frontal view (Fig. 2) shows a divided supraorbital torus, a relatively low vault and a mid-parietal swelling. The frontal bone is very slightly keeled in the sagittal plane but there are no



Fig. 2 Frontal view.

Table 1 Skull dimensions

Greatest length (glabella/opisthocranium)	—	205 mm
Greatest breadth (biparietal)	—	140 mm
Cranial index	—	68.3
Vault thickness (right and left parietomastoid and bregma)	—	12 mm



Fig. 3 Occipital view.

marked parasagittal flattenings. In occipital view (Fig. 3) the general frontal profile is confirmed but the parietal vault expansion is more clearly seen in the form of distinct parietal bossing or angulation.

The basal view (Fig. 4) of the skull shows plastic deformation which has affected the left frontal bone and to some extent the position of the left temporal bone. There are well marked frontal sinuses exposed by the loss of the ethmoids; the sinuses extend laterally into the orbital roof and posteriorly between the tables of the skull. The sphenoid is preserved in part and includes part of the body, greater wing and lesser wing of the right side and part of the body on the left. Both temporal bones are present and the right one is virtually complete. Both styloid processes are broken off but both the fossae for articulation of the mandible are present. The foramen magnum and its associated structures are entirely missing.

Both mastoid processes are limited by deeply incised mastoid notches (digastric grooves), and on the right there is a prominent occipitomastoid crest. The cranial capacity has been estimated by the water displacement of a cavity cast and the mean value of six estimates is 1,200 cm³ with a small range of observational error.

The facial fragment consists of the paired maxillary bones including the hard palate, the alveolar processes bearing some teeth as well as both zygomatic processes and the frontal process on the left. The dental arcade is U-shaped and the palate is deep

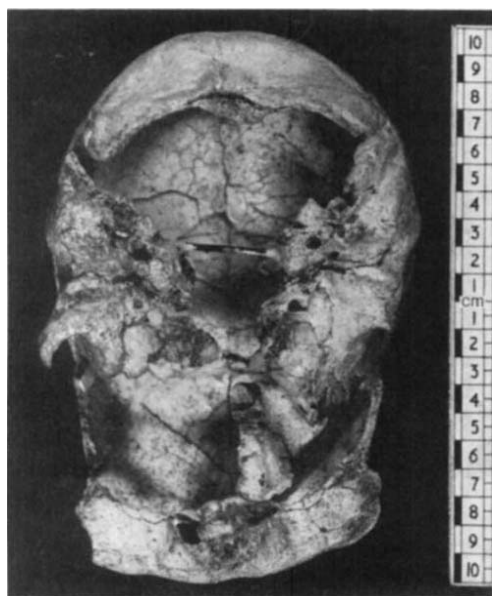


Fig. 4 Basal view.

and broad. The teeth that are present include P³, P⁴ and M¹ on the right, and the stump of P⁴ as well as M¹, M³ and M³ on the left. The dimensions of the teeth are given in Table 2. All the teeth are heavily worn, with no trace of the cusp and fissure pattern on any tooth. Dentinal exposure is present on all the molars and all the teeth have suffered from post-mortem damage. The nasal aperture is pear-shaped and there is evidence of the presence of a nasal spine.

Table 2 Dimensions of the teeth*

		1 ¹	1 ²	C	P ³	P ⁴	M ¹	M ²	M ³
Left	B/L breadth	—	—	—	—	Roots only	11.5	—	10.5
	M/D length	—	—	—	—	—	10.0	—	10.0
Right	B/L breadth	—	—	—	9.5	9.8	12.5	—	—
	M/D length	—	—	—	7.0	7.0	10.8	—	—

*All the teeth are heavily worn and damaged.

In frontal view the zygomatic processes both take off laterally well above the alveolar margin and angle sharply laterally, giving breadth to the face. In lateral view the face shows a marked degree of subnasal prognathism so that the anterior dentition would have projected to a considerable degree. The frontal process of the maxilla on the left is broken, preventing articulation of the face with the cranial vault. The distortion of the frontal bone has also made correct positioning of the two major fragments impossible for all views of the skull.

The final assessment of the affinities of the Laetoli Hominid 18 skull must await a full study including anatomical comparisons and metrical analysis. From a preliminary examination, however, there is little doubt that it should be regarded as an early East African example of sub-Saharan *H. sapiens*, of particular interest because of its good state of preservation including the presence of its facial structure. The skull shows a number of points of resemblance with the African skulls known as Omo I, Omo II (ref. 6), Broken Hill (Kabwe), Saldanha, Bodo⁷ and Ndutu^{8,9}; its anatomical features are mixed in that it has some modern characters and some that are archaic. The general expansion of the vault, the rounded form of the occiput and the low position of the inion are modern features whereas the frontal flattening, the supraorbital torus, the small mastoids, the occipitomastoid crest and its general thickness are archaic features.

The dating of the skull also adds considerably to its importance as it places the fossil near to the root of the evolution of *H. sapiens* in East Africa out of the early *H. erectus* stock represented by Olduvai Hominid 9 and KNM-ER 3733. The *H. erectus*/*H. sapiens* transition is still poorly documented despite the recent finds from East Africa, notably the Bodo skull and the skull from Ndutu. The recovery of Laetoli Hominid 18 adds to this growing sample and makes an important contribution to our understanding of the evolution of *H. sapiens* in sub-Saharan Africa.

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Aggressive tusk use by the narwhal (*Monodon monoceros* L.)

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The narwhal, an Arctic odontocete, has two horizontally embedded teeth at birth¹. In males and a few females, the left tooth erupts at the end of the first year (K. A. Hay, personal communication) and develops into a spiralled tusk, which can be up to 260 cm long. It has been suggested that the tusk is used to disturb potential benthic prey²; to pierce prey before killing³; to pierce thin ice to make breathing holes^{4,5}; as a defensive weapon⁴; as a cooling mechanism⁶, and as a sound transmitter^{7,8}; but it is most probably used in display or fighting⁹⁻¹³. There is no mention in the literature of overt aggressive behaviour between narwhals or of scars on narwhals. We have found many scars on adult male narwhals. These, together with the high incidence of broken tusks in adult males and the burst of tusk growth at sexual maturity indicate that tusks are used in aggressive encounters.

Narwhal behaviour was observed from the ice and from a cliff on northern Baffin Island in the eastern Canadian Arctic during three field seasons, from June to October of 1976-1978. Narwhal specimens, which were provided by Inuit hunters, were sampled throughout the field seasons. We measured body length, tusk length, girth and weight, and counted from photographs the number of scars visible in a frontal view on the forehead and above the mouth of each sample.

Narwhals cross their tusks^{3,9}, and strike them against one another (R. Gray cited in ref. 14). We frequently observed narwhals cross tusks above and below the water surface (Fig. 1). Tusk crossing was often accompanied by a great deal of social behaviour, which is described by Silverman¹⁵. Agonistic behaviour, indicated by abrupt and quick movements, was rare. We propose that the observed behaviour patterns are components of mating and rutting behaviour sequences. Such behaviour, which was observed outside the mating season (mating is from March to May (ref. 16 and unpublished results of K. A. Hay)), may assist juveniles to develop skills for adult sexual roles, and may also communicate dominance rank.

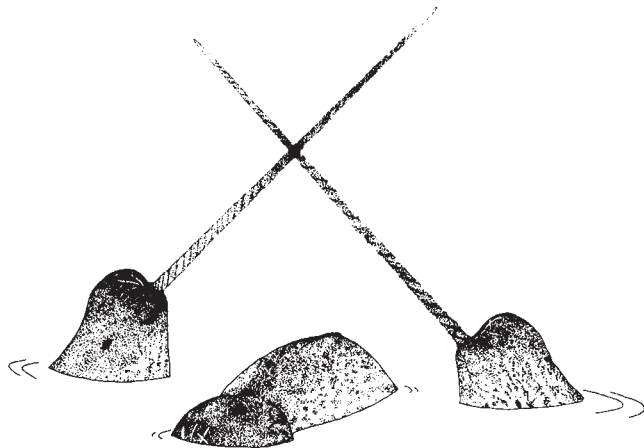


Fig. 1 Drawing of two male narwhals crossing tusks above the back of a third narwhal.

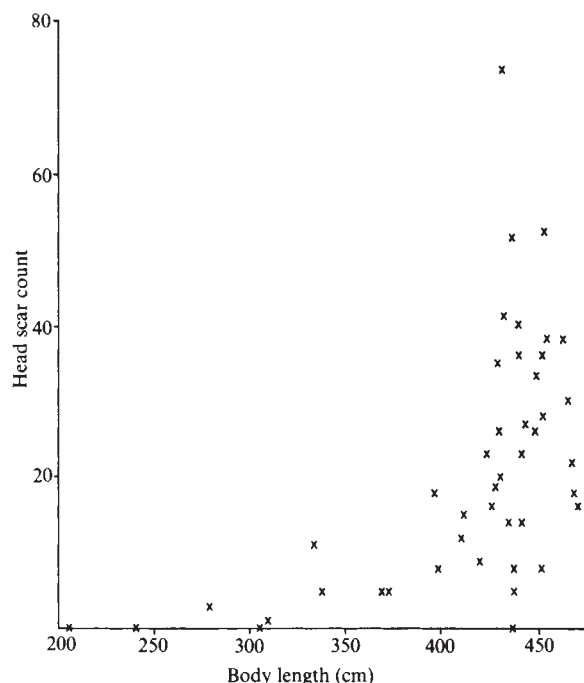


Fig. 2 Relation between body length and head scar count of male narwhals. Regression equation is: $y = 1.576 \times 10^{-12} x^{4.953}$; $r = 0.756$; y , head scar count; x , body length in cm; n , 44.

The mean number of scars on the heads of adult males was significantly greater than that on adult females ($P < 0.001$). Males become sexually mature at body lengths of about 400 cm (K. A. Hay, unpublished), at which time tusk size (girth, weight and length) increases rapidly¹⁵. Large numbers of scars on the head occur only on male narwhals longer than 420 cm, well after sexual maturity is attained (Fig. 2).

Of the 39 adult males sampled, 61.5% had broken tusks (distal end broken off), whereas only 10.3% of the 29 juvenile male samples had broken tusks. One of our samples, an adult male, had the tip of a tusk, 9 cm long, embedded in its left upper jaw beside the base of its own tusk. We observed a large scar at the site of embedding and the head was covered with scars. Only one other similar finding has been reported¹⁷. Brown¹⁸ and Porsild¹⁹ occasionally recorded broken tusks with tusk tips jammed into the pulp cavities at the sites of the breaks. Dow and Hollenberg⁶ suggested that the deposition of reparative dentine could account for the tusk 'fillings'. We agree with Porsild¹⁹ that these 'fillings' are tusk tips; the photograph in his paper is convincing. If reparative function accounted for the tusk 'fillings', they should occur more often.

Scars occur in most, if not all, odontocete species²⁰⁻²². Spacing of scars often corresponds with tooth spacing of the same species²¹. Observations of captive odontocetes show that biting and subsequent scarring occur during play, sex and aggression²³. The presence of many scars on the heads of adult males, tusk crossing behaviour, rapid tusk growth at sexual maturity, large proportion of broken tusks in adult males, and the discovery of a tusk tip embedded in the jaw of a male strongly indicate that the tusk is used in intraspecific aggression, most probably during the mating season when male-male competition is expected to be most intense.

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Synaptic mechanisms involved in responses of chromaticity horizontal cells of turtle retina

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Chromaticity-type horizontal cells^{1,2} are interneurons intervening in the first steps of colour-coding information in the vertebrate retina. In turtle retina, the most commonly found of these cells are the red/green horizontal cells (R/G-HCs)³ which are hyperpolarised by green light stimuli and depolarised by red ones. The hyperpolarisation of the R/G-HCs by green light results from a direct input from green cones which are hyperpolarised by green light⁴. The depolarisation of R/G-HCs by red light has been interpreted by Fuortes and Simon⁴ as a consequence of the depolarisation of the green cones due to the activation of a polysynaptic circuit (red cones to L-horizontal cells (L-HCs) to green cones) involving a negative feedback effect of L-HCs on the green cones. Feedback depolarisations of cones result from an increase of the cone calcium conductance which may become regenerative and give rise to spikes^{6,7}. Moreover, prolonged activation of the feedback mechanism of L-HCs on cones in the presence of Sr^{2+} ions in the extracellular medium evokes a repetitive discharge of spikes in cones⁸. In the present work, the effect of Sr^{2+} ions on the responses of both green cones and R/G-HCs have been analysed to test the Fuortes and Simon hypothesis. We have found that in Sr^{2+} media, red light stimuli covering a large retinal area elicit both a repetitive discharge of spikes in the green cones and trains of depolarising transient potentials, probably postsynaptic potentials, in the R/G-HCs. Such results support the idea that feedback depolarisation of the green cones is responsible for the depolarising responses of R/G-HCs to red light.

The experiments were carried out on eyecup retina preparations from the turtle *Pseudemys scripta elegans* placed on a suitable chamber and continuously superfused with a bicar-

bonate saline solution⁹ bubbled with a mixture of 95% O_2 + 5% CO_2 . SrCl_2 was added to the saline without molarity compensation. Intracellular recordings were made with 4 M potassium acetate-filled micropipettes (resistance 200–600 M Ω), connected through an amplifier to a cathode ray oscilloscope and to both tape and pen recorders. The retina was stimulated with a double-beam photostimulator; the light stimuli could be modified by interposition of neutral density and/or interference filters.

As Fuortes *et al.*³ previously described, stimulation with deep red lights covering large retinal fields depolarises the green cones and, in some cases, evokes an initial spike response. This is shown in Fig. 1a₁, where a green cone responded to a large spot of red (700 nm) light by a spike followed by a slight hyperpolarisation. Stimulation of the same area with a 550-nm light evoked a large hyperpolarisation (Fig. 1a₂).

Several minutes after adding 6 mM SrCl_2 to the saline bathing the retina, little change was observed in the responses to green stimuli (Fig. 1b₂), but the large red spot then evoked, almost at the dark potential level, a repetitive discharge of spikes of 25 mV showing large undershoots (Fig. 1b₁). As previously demonstrated^{6–8}, such spikes result from a regenerative increase in conductance across Ca^{2+} channels in the cone membrane and are the consequence of the activation of a feedback connection from the L-HCs⁵. As in the case of all the spikes evoked in cones through the feedback mechanism, the hyperpolarisation of the cone by either inward current injection or appropriate light stimulation can cause partial or total block of the spikes^{7,8}. This is shown for the same green cone in Fig. 1b₃ using a green light stimulus and in Fig. 1b₄ by injecting an inward current pulse.

In the Sr^{2+} -treated retina, in parallel with the appearance of spikes in green cones, trains of transient depolarising potentials were observed in the R/G-HCs. Figure 2 shows records

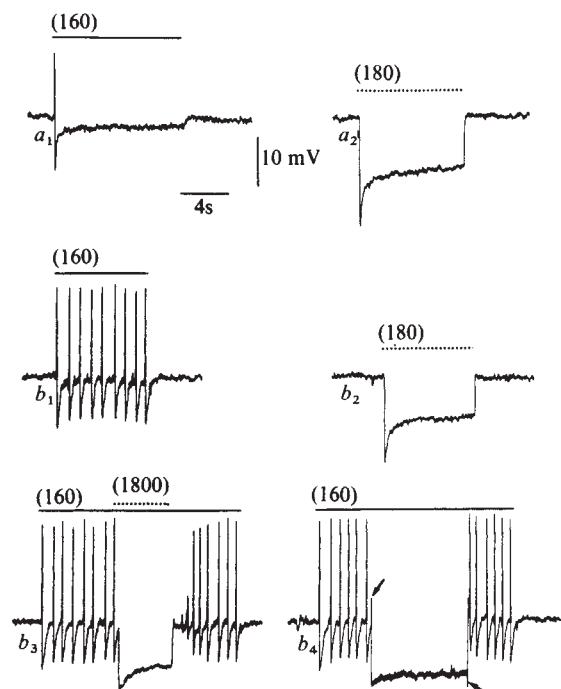


Fig. 1 Responses of a green cone to monochromatic light stimuli. In this and Figs 2 and 3, the stimuli were large spots of 3,700 μm diameter. The solid line traces above the responses indicate the duration of red (700 nm) stimuli, whereas the dotted line traces indicate the green (550 nm) stimuli. The numbers in parentheses, over the stimulus traces when multiplied by 10^3 give the quantum flux in photons $\mu\text{m}^{-2} \text{s}^{-1}$. a₁, a₂, Control recordings in normal saline. b₁–b₄, Responses of the cone during application of 6 mM Sr^{2+} . In b₄ an inward current of 0.2 nA was applied, between the arrows, through the recording microelectrode using a bridge circuit.

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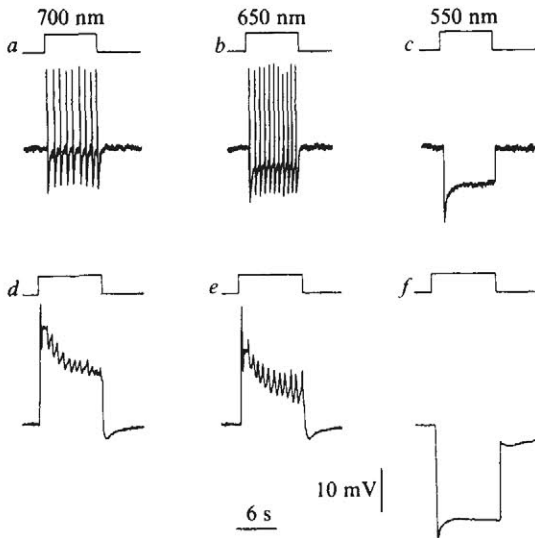


Fig. 2 Responses of a green cone (a–c) and a R/G-HC (d–f) recorded from the same retina bathed in 6 mM Sr^{2+} -containing medium. The wavelength of the light stimuli used in each aligned pair of recordings are indicated at the top. The quantum flux for all stimuli was 170×10^3 photons $\mu\text{m}^{-2} \text{s}^{-1}$.

obtained from a green cone and from a R/G-HC, recorded in the same Sr^{2+} -treated retina. Again, as in Fig. 1, large field stimulation with either 700- or 650-nm wavelength light evoked a repetitive discharge in the green cone (Fig. 2a, b). The same stimuli evoked prolonged depolarising responses in the R/G-HCs (Fig. 2d, e) consisting of an initial peak followed by a plateau phase on which was superimposed a train of transient depolarising potentials showing frequencies similar to those of the spikes in the green cones (Fig. 2d, e). In contrast, stimulation of the same retinal area with 550-nm light evoked only hyperpolarising responses in both the green cones and the R/G-HCs either before or during Sr^{2+} application (Fig. 2c, f).

Similar results were observed in 4 green cones and in 21 R/G-HCs. The frequency of the transient depolarising potentials in the R/G-HCs corresponded to that of the spike discharge in the green cones ($0.5\text{--}2 \text{ s}^{-1}$) when using the same stimuli and their amplitude varied between 2 and 10 mV. In most cases it was possible to show that the depolarisation of the R/G-HC membrane potential beyond the dark level was not necessary for the appearance of the transient depolarising potentials. This is illustrated in Fig. 3a in which large spots of 650-nm light evoked a train of depolarising potentials at a membrane potential close to the dark level. Furthermore, these depolarising transients could also be observed at membrane potentials more hyperpolarised than the dark level. Thus, when the same red stimulus was presented to the cell in the course of an hyperpolarisation elicited by a prolonged green light stimulus it evoked a large prolonged depolarising response whose plateau level did not reach the dark potential. The depolarising transients were, nevertheless, present and their amplitude even seemed to be increased (Fig. 3b). In Fig. 3c the same R/G-HC was stimulated with the same red stimulus during a rather prolonged period during which green stimuli of the same diameter, at different intensities, were intermittently combined with the red light. The two less intense green stimuli elicited hyperpolarising responses and the amplitude of the depolarising transients increased with the cell hyperpolarisation. However, the most intense green stimulus evoked a much larger hyperpolarisation and the disappearance of the depolarising potentials. It also blocked the repetitive spike discharge elicited by 650-nm light in green cones.

In two experiments using two independent microelectrodes, simultaneous recordings could be obtained from both a R/G-HC and a large-field L-HC. In the presence of Sr^{2+} in the

medium no depolarising transients could be evoked in the L-HC by stimuli which induced the appearance of transient depolarising potentials in the R/G-HC. Another point which was carefully investigated in Sr^{2+} -treated retinas was the existence of a possible relationship between the spikes that can be discharged in red cones through the activation of the feedback mechanism^{6–8} and the depolarising fluctuations evoked by red stimuli in the R/G-HC. We found that depolarising potentials in the R/G-HC could be evoked by stimuli which did not evoke spikes in the red cones, and conversely, stimuli which did evoke spikes in red cones could fail to induce the appearance of depolarising fluctuations in the R/G-HC. These results exclude any correlation between the appearance of feedback spikes in red cones and the depolarising potentials in the R/G-HC.

Our results show that in Sr^{2+} -treated turtle retinas, red light stimuli which elicit repetitive discharge of spikes through the feedback mechanism in green cones also evoke the appearance of depolarising potentials in the R/G-HCs. The spikes in the green cones are due to a regenerative increase in conductance through Ca^{2+} -membrane channels.

What is the nature of the depolarising transient potentials observed in the R/G-HCs? It seems unlikely that these depolarising potentials could be regenerative responses triggered by the sustained depolarisations evoked by red light in the R/G-HCs because such sustained depolarisations are not necessary for the depolarising transients to appear, and because these deflections can be elicited at membrane potentials more hyperpolarised than the dark potential. In contrast, many of the features of the depolarising potentials in the R/G-HCs are consistent with the idea that they are postsynaptic potentials due to phasic transmitter release evoked by the spikes in the green cones. The depolarising transients only appear in Sr^{2+} -treated R/G-HCs and only in response to stimuli which evoke spike discharge in the green cones, the trains of depolarising potentials in R/G-HCs showing a frequency similar to those of the cone spikes and becoming blocked by light stimuli which suppress the spikes in the green cones. Moreover, when the R/G-HCs are hyperpolarised by light stimuli which do not block the spikes in green cones, the amplitude of the depolarising potentials increase. Such behaviour is reminiscent of the classical depolarising synaptic potentials.

The present experiments, therefore, strongly suggest that the depolarising deflections evoked in the Sr^{2+} -treated R/G-HCs

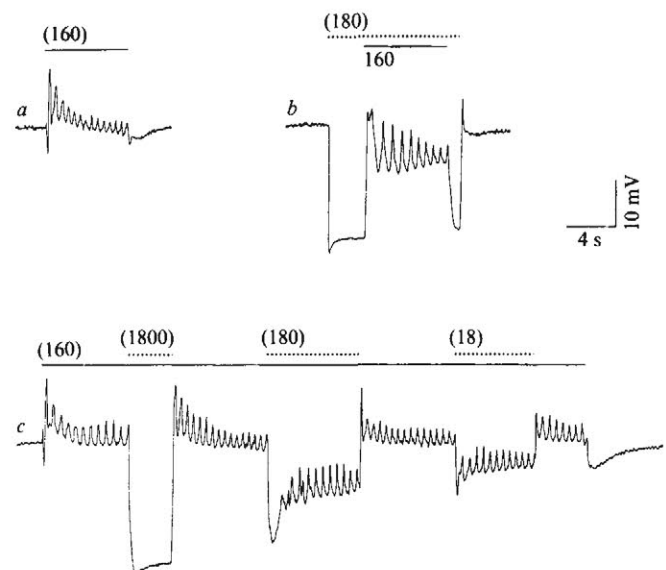


Fig. 3 Responses from a R/G-HC recorded in a retina bathed in a 6 mM Sr^{2+} -containing medium. The full line traces above the recordings indicate the duration of red (650 nm) stimuli whereas the dotted lines indicate the duration of green (550 nm) stimuli. The numbers in parentheses, when multiplied by 10^3 give the quantum flux in photons $\mu\text{m}^{-2} \text{s}^{-1}$.

by red lights are depolarising synaptic potentials secondary to presynaptic spikes evoked through the feedback mechanism of L-HCs on the green cones.

What is the relevance of these results obtained in Sr^{2+} -treated retinas to the known properties of the R/G-HC responses in the normal retina? According to Fuortes and Simon, (1) R/G-HCs receive their only direct input from the green cones; (2) a green stimulus hyperpolarises the green cones, then the R/G-HC; (3) green cones are almost unaffected by red light impinging on them; (4) red illumination of a large area of the retina hyperpolarises the red cones, thus hyperpolarising the L-HCs which receive their main input from red cones; (5) the L-HCs send back their signal on to the green cones, depolarising them, in consequence depolarising the R/G-HCs. The recent anatomical studies of Leeper^{10,11} are consistent with this hypothesis. This author identifies the R/G-HCs with one morphological type of horizontal cell (H2) which is mainly in contact with green cones, and describes another type of horizontal cell (H1-CB) which would be responsible for the feedback effects on the green cones.

From the known properties of photoreceptor synapses¹²⁻¹⁶, hyperpolarisation by green light is expected to suppress the transmitter release from green cones whereas their feedback depolarisation by red light, which involves an increase in Ca^{2+} conductance⁶⁻⁸, is expected to increase the release of transmitter. Our results thus give further support to the hypothesis of Fuortes and Simon, as they indicate that stimuli which evoke depolarising responses in R/G-HCs cause a powerful activation

of the feedback circuit affecting the green cones and when this feedback activation gives rise to a repetitive discharge of Ca^{2+} spikes, depolarising synaptic potentials are actually observed in R/G-HCs. Therefore, the release of a single transmitter from green cones, depressed by the hyperpolarisation evoked by green light and increased by the red light activation of the feedback circuit, can account for the opposite polarity of the responses of the R/G-HCs to such stimuli.

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Postsynaptic effects of ethanol at the frog neuromuscular junction

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The molecular mode of action of alcohol in the central nervous system (CN) is unclear¹. The effects of ethanol on axonal action potentials can only be measured at concentrations which are very much higher than those required to produce central effects². At the frog neuromuscular junction similar concentrations increase the open time (τ) of the ion channel associated with the nicotinic acetylcholine (ACh) receptor³. We have now investigated the effect of ethanol on the postsynaptic membrane of the frog neuromuscular junction by measuring equilibrium dose-response curves for the interaction between the neurotransmitter (ACh) and the ACh receptors. Using this system, we found that ethanol produces significant changes in receptor function. Moreover, we found that at an ethanol concentration which can be physiologically tolerated by man (0.2%) the dose-response curve is measurably affected.

The neuromuscular junction of the cutaneous pectoris muscle in the frog (*Rana esculenta*) is ideal for such studies⁴. The muscle can be dissected to a monolayer of fibres⁵, and the endplate region of the cell membrane which contains the nicotinic ACh receptors can be readily detected with Nomarski optics⁶. The cell membrane is voltage clamped by a conventional two-electrode system and an iontophoresis pipette for the experimental delivery of ACh is placed at a known distance (z) above the finger-like endplate area. A current pulse of known duration and amplitude is applied to the pipette, resulting in the release of a controlled amount of ACh from the tip⁷. The ACh

diffuses to the receptors, thus giving rise to a transient increase in membrane current which is due to the opening of ion channels associated with the receptors^{8,9}. In defined conditions it can be shown that the response is in equilibrium with the concentration of ACh at the receptor site^{10,11}.

Figure 1a shows a typical current response produced by an iontophoretic pulse of ACh in three concentrations of ethanol at the same endplate. The peak current (I_p) in this response was almost doubled in the presence of only 0.2% ethanol. The time to peak (t_p) did not change with the application of ethanol, indicating that saturation of the endplate receptors had not been reached¹⁰. In this condition it was also possible to determine the Hill coefficient (n_H) from the actual time course of the ACh response¹⁰. Such an analysis showed that ethanol had no effect on the value of n_H .

If, in an experiment like that shown in Fig. 1a, one plots the peak amplitude of the response (I_p) against the charge Q (duration multiplied by amplitude of releasing pulse) for different charges, then a dose-response curve is obtained (Fig. 1b). These curves are highly reproducible^{11,12}. From the charge through the pipette it is possible to calculate the amount of ACh which is released and by use of the diffusion equation the concentration profile at the endplate may be defined. From the equilibrium dose-response curve the following parameters may be calculated: (1) the maximum conductance, $g_{\max}$, per unit length of the terminal (in $\text{nS } \mu\text{m}^{-1}$); (2) the apparent dissociation constant K (in μM) which reflects both the binding and the conformational change of the drug-agonist interaction; (3) the Hill coefficient, n_H . These three parameters completely describe the equilibrium reaction between ACh and the receptors. The evaluation of the parameters of equilibrium dose-response curves is dependent on the use of a kinetic model for the drug-receptor interaction. The model and procedure used here have been explicitly described elsewhere¹², and for an alternative method see ref. 13.

Figure 1b shows a typical ACh dose-response curve obtained before and after the addition of 2% ethanol. The solid lines are the standard curves which provide the best fit to the data. The curve is shifted to the left, signifying that a fixed dose of ACh has a greater effect in the presence of ethanol. It can be seen that the

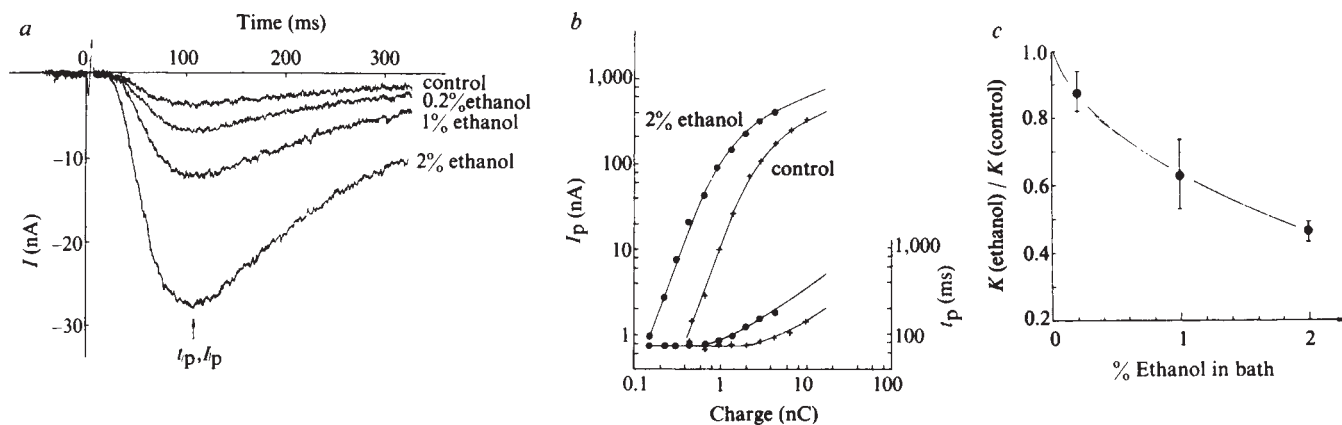


Fig. 1 *a*, Influence of different concentrations of ethanol on endplate currents produced by iontophoretic release of ACh from a pipette located 25 μm above a single linear endplate terminal. The two clamping electrodes were placed on either side of the endplate close to the ACh pipette. The iontophoretic pulse was initiated at time 0. The pulse duration was 1.5 ms and the pulse height 300 nA (charge: 0.45 nC). Ethanol (Merck, 96 vol %) at the above concentrations was diluted in Ringer solution and exchanged into the bath for each measurement. All measurements were obtained at the same endplate in identical conditions. The time to peak (t_p) for each response was unchanged, signifying that the ACh pipette always remained at the same location. The Ringer solution had the following composition (mM): NaCl 115, KCl 2.5, CaCl_2 1.8, phosphate buffer 5, pH 7.1. The membrane potential was -80 mV and the bath temperature was 18°C . The effects of ethanol at any concentration could be reversed by returning to normal Ringer solution whereupon the maximum current (I_p) of the response returned to the control value. *b*, ACh dose-response curves at a single endplate before and after the addition of 2% ethanol to the Ringer solution. The maximum current (I_p) for each response and the associated time to peak (t_p) were plotted against the charge passed through the ACh-releasing pipette (log-log scale). The membrane potential was -80 mV and the temperature was 18°C . The Hill coefficient, n_H (2.8), was measured from the slope of the line at low drug concentrations and was not changed by the addition of 2% ethanol. The solid lines are the theoretically derived curves ($n_H = 2.8$) which permit the calculation of g_{max} and K . The time (t_p , lower trace) to peak current (I_p , higher trace) remains constant for low doses and increases at higher doses. The parameters for the control dose-response curve were n_H 2.8, g_{max} $176 \text{ nS } \mu\text{m}^{-1}$, K $36 \mu\text{M}$, and for 2% ethanol n_H 2.8, g_{max} $168 \text{ nS } \mu\text{m}^{-1}$, K $13.6 \mu\text{M}$ (see Table 1). *c*, Comparison of the apparent dissociation constant K between controls and different concentrations of ethanol for ACh dose-response curves. Each K value was computed for a control dose-response curve and a dose-response curve after the addition of ethanol measured at the same endplate. The ratios were averaged for several different endplates and the graph shows the respective mean values $\pm$ s.e.m. (see Table 1).

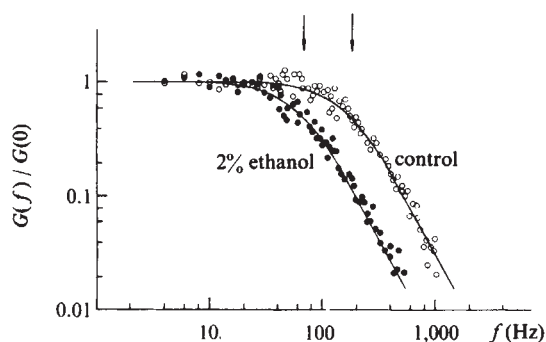


Fig. 2 Normalised power spectrum (log-log scale) calculated from ACh-induced endplate current noise before and after the addition of 2% ethanol at the same endplate. The ACh pipette was located $70 \mu\text{m}$ above the endplate and ACh was continuously released, resulting in a constant mean endplate current, μ_I . The resulting noise was filtered between 3 and 1,000 Hz, digitised at 2,000 Hz and stored in frames of 1,024 points. The power spectrum was averaged from 20 frames. In each case a background control spectrum in the absence of ACh was subtracted from the ACh-induced spectrum. The solid lines are the theoretical lorentzian functions which provide the best fit to the data. The mean channel lifetime (τ) was obtained from each curve by the formula $\tau = 1/(2\pi f_c)$, where f_c is the cutoff frequency designated by the arrows above each curve. The single channel conductance was obtained from the formula $\gamma = G(0)/[4\mu_I(V_m - V_{\text{eq}})\tau]$, where V_{eq} is the ACh null potential (-5 mV), V_m is the membrane potential (-80 mV) and $G(0)$ is the zero frequency amplitude. The temperature was 18°C . For the control spectra above, $G(0) = 1.67 \times 10^{-22} \text{ A}^2 \text{ Hz}^{-1}$, $\mu_I = -26 \text{ nA}$, $f_c = 174 \text{ Hz}$, $\tau = 0.92 \text{ ms}$ and $\gamma = 23.4 \text{ pS}$. For the spectra in 2% ethanol, $G(0) = 4.39 \times 10^{-22} \text{ A}^2 \text{ Hz}^{-1}$, $\mu_I = -29 \text{ nA}$, $f_c = 66 \text{ Hz}$, $\tau = 2.4 \text{ ms}$ and $\gamma = 20.9 \text{ pS}$. Noise analysis at four different endplates before and after the addition of 2% ethanol showed that γ was reduced to 0.91 ± 0.06 (s.e.m.) of the control value and τ was increased to 2.2 ± 0.14 (s.e.m.) of the control value.

slopes of both curves are identical, and thus that the Hill coefficient or cooperativity of the receptor response to ACh is unchanged. This value of 2.7 implies that three or more (perhaps four) ACh molecules are required to bind for one channel to open. The value of K was dramatically reduced, as is evident from the shift in the dose-response curve. In Fig. 1c the relative change in K is plotted against the ethanol concentration, showing that at the lowest concentration of 0.2%, a reduction of 10–20% in K is produced, corresponding to a doubling of the response in the linear part of the dose-response curve.

g_{max} Was not affected by 0.2% or 1% ethanol but was slightly reduced by 2% ethanol (Table 1) which would reflect either a reduction in the total number of channels capable of opening or in the size of the individual channel. This was tested by noise analysis, which showed that indeed the individual channel conductance (γ) was reduced by about 9% and that, in addition, the lifetime of the single channel (τ) was increased in 2% ethanol (Fig. 2). These changes in single channel properties are similar to the findings of Gage *et al.*³. The reduction of γ could, therefore, account for the slight reduction in g_{max} which we have observed in the presence of 2% ethanol.

We therefore conclude that the drastic increase of the post-synaptic response in ethanol as shown in Fig. 1a is due to a reduction in K . Note that even a small change in K will result in a large change in I_p for a constant Q . This effect is due to the steep dependence of I_p on Q , the slope being equal to the Hill coefficient. However, with our method we cannot discriminate between whether only the conformational change or the binding step in the drug-receptor interaction or both have been affected by ethanol.

Dose-response curves are carried out using carbachol as agonist or with ACh after the inhibition of esterase by edrophonium gave a similar reduction in K in the presence of ethanol (Table 1). This effect of ethanol is, therefore, not due to an inhibition of cholinesterase, which could change the apparent dissociation constant. Note that the reduction of K in the

Table 1 Ratio of $g_{\max}$ and K values $\pm$ s.e.m. obtained in different concentrations of ethanol to control values for ACh and carbachol as agonist

Drug	$\frac{g_{\max}(\text{ethanol})}{g_{\max}(\text{control})}$	$\frac{K(\text{ethanol})}{K(\text{control})}$
Acetylcholine		
0.2% ethanol	1.00 $\pm$ 0.08 (4)	0.88 $\pm$ 0.06 (4)
1.0% ethanol	0.97 $\pm$ 0.07 (3)	0.63 $\pm$ 0.10 (3)
2.0% ethanol	0.93 $\pm$ 0.03 (8)	0.46 $\pm$ 0.03 (8)
2.0% ethanol		
+5 μ M edrophonium	0.92 $\pm$ 0.06 (2)	0.48 $\pm$ 0.04 (2)
Carbachol		
2% ethanol	0.92 $\pm$ 0.06 (6)	0.62 $\pm$ 0.06 (6)

For each data point a dose-response curve was obtained at the same endplate before and after the addition of the corresponding concentration of ethanol. The average values for the parameters of the control dose-response curves were for ACh: $n_H = 2.7$, $g_{\max} = 169 \text{ nS } \mu\text{M}^{-1}$, $K = 27.8 \text{ } \mu\text{M}$ (corrected for ACh-esterase activity) and for carbachol: $n_H = 2.2$, $g_{\max} = 150 \text{ nS } \mu\text{M}^{-1}$, $K = 336 \text{ } \mu\text{M}$ (ref. 10). Numbers in parentheses refer to the number of fibres investigated.

presence of ethanol is greater with ACh as the agonist than with carbachol. As already reported¹², control dose-response curves for carbachol give a lower Hill coefficient, a lower $g_{\max}$ and a higher K than ACh (Table 1). Also, the reduction in $g_{\max}$ in the presence of 2% ethanol is the same as for ACh as for carbachol, showing that the efficacy¹² of carbachol in relation to ACh is not changed.

A reduction of the dissociation constant K by ethanol concentrations as low as 0.2% (a value which can be found in an intoxicated human) could also be demonstrated on mammalian endplates (preliminary experiments on rat and mouse omohyoideus, not illustrated). Here the alcohol effect is even more pronounced.

Ethanol cannot be expected to improve neuromuscular transmission which already has a high safety factor, in healthy specimens. However, it may very well have a positive effect in patients in whom the neuromuscular transmission is impaired (for example, in cases of myasthenia gravis). This positive effect of ethanol could indeed be demonstrated in stimulation electromyography of myasthenic patients, where ethanol concentrations as low as 0.05% proved to be effective¹⁴.

One might speculate that the ability of ethanol to improve the binding of ACh to neurotransmitter receptors could also have highly significant effects in central synapses and thus influence intraneuronal communication which gives rise to normal behaviour.

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Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue

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The hypothalamus has been implicated in the development of obesity in both humans¹ and experimental animals². The syndrome of hypothalamic obesity results from destruction of the ventromedial region of the hypothalamus³, and investigations of the influences of the hypothalamus on lipid metabolism have been directed chiefly at understanding the mechanism underlying obesity^{2,4,5}. There have been few studies of the effects on lipolysis and lipogenesis in adipose tissue after stimulation of the ventromedial and lateral hypothalamic (LH) nuclei, which may act reciprocally in regulating certain types of peripheral metabolism⁶⁻⁹. We have shown¹⁰ that electrical stimulation of the ventromedial hypothalamic nucleus (VMH) causes lipolysis in adipose tissue, detected by a rapid rise in the plasma glycerol concentration, whereas electrical stimulation of the LH has no such effect. It is not known whether hypothalamic stimulation of either the VMH or the LH affects lipogenesis in adipose tissue. We have investigated this problem by measuring the incorporation of tritium from ³H₂O into fatty acids *in vivo*. This is the most reliable method for measuring the rate of fatty acid biosynthesis, because it is independent of the carbon precursor^{11,12}. Surprisingly, electrical stimulation of the VMH enhanced fatty acid synthesis in brown adipose tissue, but not in white adipose tissue or the liver, without the intervention of insulin secretion. Electrical stimulation of the LH, however, had no appreciable effect on lipid synthesis in either type of adipose tissue.

Female Wistar rats weighing about 230 g were housed in individual cages and given free access to laboratory chow and water. The hypothalamus was stimulated, through chronically implanted electrodes, without disturbing the animals' behaviour. Bipolar electrodes of insulated 160- μ m wire were implanted stereotactically and unilaterally in the VMH or the LH of rats under pentobarbital anaesthesia. The electrodes were connected to a small plug which was anchored firmly to the skull⁷. The position of the tip of the electrode was verified microscopically in brain sections made when the experiments were complete. Two weeks after implantation, electrical stimuli, consisting of monophasic square pulses of 0.1-0.3 ms duration at 100 Hz with an amplitude of 6 V, were applied to the hypothalamus. Control rats had similarly implanted electrodes but no electrical stimuli were applied. An interval timer was connected to the stimulator to allow repeated stimulation for 30-s periods, once every minute. After 10 min of intermittent hypothalamic stimulation, the rats were injected with ³H₂O and 30 min later they were killed. The interscapular adipose tissue (brown fat), the parametrial and retroperitoneal adipose tissues (white fat), and the liver were removed and frozen on dry ice. Total lipids from each tissue were hydrolysed, and after extraction of the fatty acids radioactivity was measured (Fig. 1). In later experiments, some rats were made diabetic (blood glucose levels at the time of death >400 mg dl⁻¹) by intravenous injection of streptozotocin (65 mg per kg) 24-26 h before hypothalamic stimulation. To minimise the effects of environmental temperature¹³ and of circadian variation¹⁴ on lipogenic activity in adipose tissue, all experiments were performed at 25 $\pm$ 2 °C and between 10.00 h and noon, and the animals were not fed for 3 h before the experiments.

The rate of fatty acid synthesis in control rats was about four times greater in brown (interscapular) adipose tissue than in white (parametrial and retroperitoneal) adipose tissue (Fig. 1). With electrical stimulation of the VMH, the rate increased more (about 2.3 times that of unstimulated controls) in the interscapular adipose tissue ($P < 0.05$), but not in the white adipose tissue. Slightly increased rates observed in parametrial and retroperitoneal adipose tissue were not statistically significant ($P > 0.05$). Electrical stimulation of the LH did not appreciably affect lipid synthesis in either type of adipose tissue. Fatty acid synthesis in the liver was not affected by stimulation of the VMH or the LH.

The differential effects of VMH stimulation on lipogenesis in brown and white adipose tissues are probably not mediated by increased secretion of insulin, a pancreatic hormone that stimulates lipid synthesis, because electrical stimulation of the VMH suppressed rather than increased insulin secretion^{8,15}, and if insulin output had increased, fatty acid synthesis would have been enhanced equally in brown and white adipose tissues and liver. When rats were injected with insulin (0.2 U, intraperitoneally), a marked increase in tritium incorporation into fatty acids was observed consistently in all three tissues (data not shown). Table 1 shows the effects of injury to the pancreatic β cells by intravenous injection of streptozotocin before stimulation of the VMH. The appearance of hyperglycaemia (more than 400 mg dl⁻¹) and glucosuria confirmed that insulin secretion was suppressed.

The rate of fatty acid synthesis in the two types of adipose tissue and in the liver of diabetic control rats was reduced to below half the values in healthy control rats, possibly due to the absence of the lipogenic action of insulin. Nonetheless, electrical stimulation of the VMH of the diabetic rats markedly increased lipid synthesis in brown adipose tissue ($P < 0.05$). Again, there was no significant increase in the lipogenic response in either

Table 1 Lipogenic responses of adipose tissues and liver to stimulation of VMH in streptozotocin-induced diabetic rats

	Diabetic control	Diabetic with VMH stimulation
Interscapular adipose tissue	0.48 ± 0.04	1.11 ± 0.12*
Retroperitoneal adipose tissue	0.15 ± 0.01	0.19 ± 0.01
Parametrial adipose tissue	0.16 ± 0.02	0.20 ± 0.02
Liver	0.48 ± 0.05	0.37 ± 0.04

Experimental conditions were as for Fig. 1, except that rats were made diabetic by injection of streptozotocin (65 mg kg⁻¹, intravenously) before they were stimulated electrically. Incorporation of tritium into fatty acids extracted from the adipose tissues and liver was measured, and the rate of fatty acid synthesis was expressed as $\mu\text{mol per g wet weight of tissue in 30 min}$. Results are means $\pm$ s.e.m. of the values in eight animals in each group.

* Significantly different from the unstimulated control group at $P < 0.05$.

retroperitoneal or parametrial white adipose tissue, and conversely fatty acid synthesis in the liver decreased slightly, though not significantly ($P > 0.05$), with stimulation of the VMH.

These results indicate that stimulation of the VMH enhances lipogenesis in brown adipose tissue preferentially, through a mechanism not involving insulin. This effect is probably mediated by sympathetic innervation of brown adipose tissue, because the VMH is presumed to be part of the sympathetic neural output from the hypothalamus^{8,16}. Brown adipose tissue has abundant sympathetic innervation with adrenergic fibres, which form nest-like networks around every fat cell¹⁷. On the other hand, white adipose tissue contains no adrenergic fibres except those related to the blood vessels¹⁸, and consequently it contains much less noradrenaline than does brown adipose tissue. Therefore, noradrenaline may be a mediator of the effect of VMH stimulation on lipogenesis in brown adipose tissue. This idea is supported by preliminary results showing that intraperitoneal injection of noradrenaline (3×10^{-7} mol) into rats increased the rate of fatty acid synthesis in interscapular adipose tissue. However, it is also possible that some lipogenic factor(s) released from the hypothalamus acts on brown fat cells; such a factor has been found in an extract of bovine hypothalamus¹⁹.

Our results, together with the previous observation of increased lipolysis in adipose tissue after stimulation of the VMH¹⁰, suggest that both the breakdown and resynthesis, that is, the turnover, of triglycerides in brown adipose tissue, but not in white adipose tissue, are accelerated by stimulation of the VMH. Because brown adipose tissue is an important fat depot for physiological lipolysis mediated by the sympathetic nerves, especially during cold acclimatisation, this could also explain the increased lipid turnover in the brown adipose tissue of cold-acclimatised animals^{20,21}.

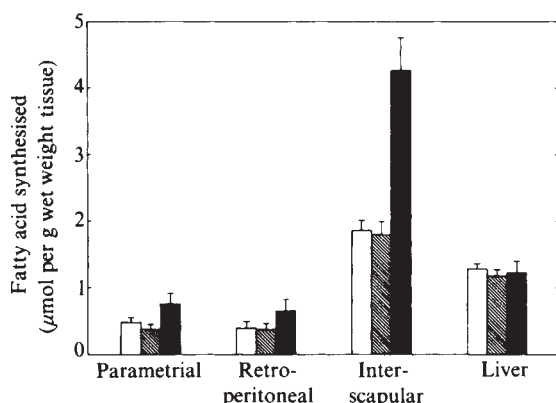


Fig. 1 Effect of hypothalamic stimulation on fatty acid synthesis *in vivo* in adipose tissues and liver. The VMH or LH was stimulated intermittently for 10 min and then the rats were injected intraperitoneally with 3 mCi of $^3\text{H}_2\text{O}$ in 0.3 ml of 0.9% NaCl solution. After another 30 min period of stimulation, the rats were killed and the interscapular, retroperitoneal and parametrial adipose tissues and the liver were removed and frozen rapidly, and the incorporation of tritium into their fatty acids was measured. Lipids were extracted by homogenising a portion (1 g) of each frozen tissue with 19 ml of chloroform-methanol (2:1, v/v). For isolation of radioactive fatty acids, the lipid extract was washed once with 0.13 M NaCl solution and three times with methanol-0.13 M NaCl-chloroform (50:47:3, by vol), evaporated under nitrogen, saponified with 5 N NaOH at 80°C for 3 h, acidified with 10 N H_2SO_4 , and then extracted three times with petroleum ether. The final extract was evaporated and its radioactivity was counted in toluene scintillation solution¹². The rate of fatty acid synthesis was calculated from the radioactivity in fatty acids and in the plasma obtained at the time of death¹⁴. Columns and bars are means and standard errors of values in six to eight animals. Open columns, controls; hatched, LH; solid, VMH.

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New class of glutamate agonist structurally related to ibotenic acid

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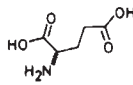
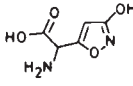
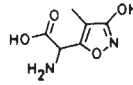
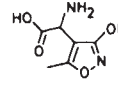
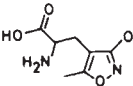
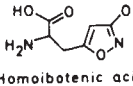
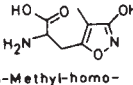
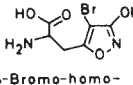
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L-Glutamic acid (Glu) and L-aspartic acid (Asp) are putative excitatory transmitters in the mammalian central nervous system (CNS)¹⁻³. Receptors at Glu- and Asp-mediated synapses are presumably different^{4,5}, and a prerequisite for the identification and characterisation of such sites is the availability of specific antagonists and agonists. Among various potential Glu and Asp antagonists³⁻⁶ Glu diethyl ester (GDEE)⁷⁻⁹ and (D)- α -aminoadipic acid (α -AA)⁹⁻¹³ show some selectivity, the latter particularly towards excitation by *N*-methyl-Asp. Kainic acid (KA), a structural analogue of Glu, is a powerful excitant of CNS neurones¹⁴⁻¹⁶ that seems to interact with only a small proportion of Glu receptors⁵. Ibotenic acid (Ibo) is a powerful neuronal excitant^{9,17,18} also structurally related to Glu. Excitation by Ibo, however, is readily antagonised by α -AA, whereas GDEE has little or no effect¹³, suggesting that Ibo preferentially activates Asp rather than Glu receptors. Furthermore, excitation of neurones by Ibo is followed by a prolonged depression of excitability^{18,19} which is sensitive to bicuculline methochloride¹⁹, indicating that Ibo is probably converted by decarboxylation into muscimol²⁰ during microelectrophoretic ejection near CNS neurones. Thus, neither KA nor Ibo seem to have sufficient specificity to be useful compounds with which to study central Glu or Asp receptors. We describe here a new class of Glu agonist obtained by structural manipulation of Ibo (Table 1). Elongation of the side chain of Ibo by an additional methylene group and introduction of different ring substituents have led to isoxazole amino acids with carboxyl groups resistant to decarboxylation. A further aim of this homologation was to convert the apparent Asp agonist Ibo into a Glu agonist.

Experiments were carried out on lumbar dorsal horn interneurons and Renshaw cells of 11 cats anaesthetised with pentobarbitone sodium (35 mg per kg body weight intraperitoneally, supplemented when required). Extracellular action potentials were recorded by means of the centre barrel of seven-barrel micropipettes, and the compounds tested were administered electrophoretically as anions from aqueous solutions in the outer barrels of the micropipettes²¹. The approximate potency of each excitant was assessed relative to that of Glu on the basis of the electrophoretic current required to produce equal and submaximal excitatory effects on 3 to 19 neurones, taking into account the addition of NaCl (100–165 mM) to dilute solutions (10–50 mM) of strong excitants. Antagonism of amino acid excitation by GDEE and α -AA was tested in each case on 2 to 9 neurones as described previously^{12,13} using Glu and *N*-methyl-(D)-aspartic acid (NMDA) as reference excitants. As in these studies antagonism by either GDEE or α -AA was rarely completely specific, and in Table 1 'yes' indicates a consistent and significantly greater reduction of the effects of a particular excitant by one of the antagonists compared with the other.

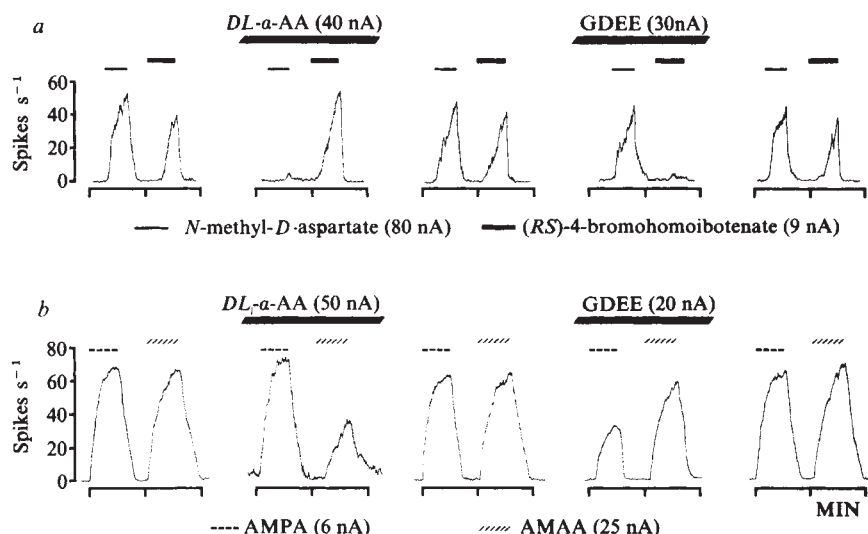
* To whom correspondence should be addressed.

Table 1

COMPOUND	EXCITATION OF		INHIBITION OF	
	CAT	SPINAL NEURONES	KAINIC ACID BINDING	
	Rel. potency	Antagonism by GDEE	α -AA	IC ₅₀ values μ M
 (S)-Glutamic acid	++	Yes		0.41
 Ibotenic acid	++++		Yes	12.2
 (RS)-4-Methyl-ibotenic acid	++(+)		Yes	10.0
 (RS)- α -Amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid (AMAA)	++		Yes	>100
 (RS)- α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)	+++++	Yes		>100
 (RS)-Homoibotenic acid	+(+)	Yes		>100
 (RS)-4-Methyl-homo-ibotenic acid	+++	Yes		>100
 (RS)-4-Bromo-homo-ibotenic acid	++++	Yes		>100

The relative potency of the amino acids as excitants was determined microelectrophoretically as described in the text and shown in Fig. 1. The effects of the excitants are expressed relative to that of Glu (++) by the number of symbols indicating lower, equal, or greater activity, and (+) being of less value than +. Antagonism of the excitatory effects of the amino acids by GDEE or α -AA is indicated by 'yes'. For the ³H-KA binding assay procedure aliquots of rat brain synaptic membranes (0.8–1.2 mg of protein), isolated by a described procedure²³, were incubated in triplicate at 4 °C for 20 min in 2 ml 50 mM-Tris-citrate buffer (pH 7.1), which was 40 nM with respect to ³H-KA, and which contained varying concentrations of KA, Glu, or the listed isoxazole amino acids. After incubation the reaction was terminated by centrifugation for 10 min at 48,000g. The supernatant fluid was discarded, and the pellet was rinsed with two 5-ml portions of ice-cold distilled water. The pellet was suspended in 1 ml of water. To 0.8 ml of this suspension was added 10 ml of scintillation liquid (Insta-gel, Packard), and radioactivity was measured in a liquid scintillation counter (Beckman LS 250). Total specific ³H-KA binding (1,500 c.p.m. per mg of protein) was obtained by subtracting from the total bound radioactivity in the absence of inhibitor (2,225 c.p.m. per mg of protein) the amount not displaced by 100 μ M KA (725 c.p.m. per mg of protein). IC₅₀ values were estimated by examining the effects of at least four different concentrations of the inhibitor and performing log-probit analyses of the results. Log-probit analyses³⁰ and protein measurements³¹ were carried out using published procedures.

Fig. 1 The effects of GDEE and DL- α -AA on the excitation of the firing of two spinal interneurons by NMDA, (RS)-4-bromo-homoibotenic acid, AMPA and AMAA. *a*, NMDA (80 nA, 50 mM in 165 mM NaCl, pH 7), (RS)-4-bromo-homoibotenic acid (9 nA, 200 mM, pH 7), DL- α -AA (40 nA, 200 mM, pH 7), GDEE (30 nA, 200 mM, pH 3.5), all ejected microelectrophoretically as indicated by the appropriate horizontal lines. *b*, AMPA (6 nA, 200 mM, pH 7), AMAA (25 nA, 200 mM, pH 7), DL- α -AA (50 nA, 200 mM, pH 7), GDEE (20 nA, 200 mM, pH 3.5). Ordinates: firing rate, spikes s^{-1} . Abscissae: time, min.



The compounds listed in Table 1 were also tested as inhibitors of the sodium-independent binding of 3H -KA²² to synaptic membranes isolated from rat brains as previously described²³. (RS)-Homoibotenic acid and (RS)-4-bromo-homoibotenic acid²⁴, (RS)- α -amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid (AMAA)²⁵, and (RS)-4-methyl-homoibotenic acid²⁶ were prepared by published procedures. The synthesis of the new compounds (RS)-4-methyl-Ibo and (RS)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (see Table 1) will be published elsewhere.

The excitatory action of 4-methyl-Ibo was weaker than that of Ibo and slightly more potent than that of Glu. The effects of Ibo and 4-methyl-Ibo on all three neurones examined were reversibly antagonised by α -AA, whereas GDEE had little or no antagonistic effect. Excitation by 4-methyl-Ibo did not seem to be followed by depression, but as the corresponding decarboxylated compound, 4-methyl-muscimol, is a relatively weak neuronal depressant²⁷, *in vivo* production of this compound may not have been detectable in these experiments. Whereas homoibotenic acid was slightly weaker than Glu as an excitant, its ring-substituted derivatives were much more potent (Table 1). Thus, 4-bromo-homoibotenic acid was more potent than Ibo, which in turn was more potent than 4-methyl-homoibotenic acid. The excitatory effects of homoibotenic acid, 4-bromo-homoibotenic acid, and 4-methyl-homoibotenic acid were completely or partially antagonised by GDEE in a reversible way, but in no case were the excitatory effects of these compounds significantly reduced by α -AA (Fig. 1*a*).

Similarly, AMPA was a GDEE-sensitive, α -AA-insensitive neuronal excitant and was even more potent than 4-bromo-homoibotenic acid (Fig. 1*b*). Whereas AMPA is a Glu analogue, its lower homologue AMAA is structurally related to Asp. Accordingly, the excitatory effect of AMAA, which was similar in potency to that of Glu, was selectively reduced by α -AA. In

general, the neuronal excitations induced by the isoxazole amino acids listed in Table 1 were prolonged compared with that of Glu, suggesting that these compounds were not as effectively removed from the vicinity of the neurones as is Glu. Furthermore, depression was observed only after excitation by Ibo.

In agreement with previous findings, KA^{22,28,29} was found to be a potent inhibitor of 3H -KA binding with an IC_{50} of 0.013 μ M, whereas Glu and in particular Ibo were much weaker (Table 1). With the exception of 4-methyl-Ibo, which like Ibo was a weak inhibitor of 3H -KA binding, none of the isoxazole amino acids studied had any effect in this test system.

These *in vitro* and *in vivo* results suggest that AMPA, homoibotenic acid and its 4-methyl and 4-bromo derivatives excite cat spinal neurones by activating Glu receptors which are different from those on rat brain membranes which bind KA, and that this series of compounds may be useful for future pharmacological analyses of CNS Glu receptors.

In contrast to AMPA, in which the negative charges are separated by five carbon atoms, homoibotenic acid and its derivatives are not strict Glu analogues. Nevertheless, all of these heterocyclic amino acids seem to act on the same receptor, and introductions of ring substituents into homoibotenic acid result in much more potent excitants. The side chains of AMPA and of 4-methyl- and 4-bromo-homoibotenic acid are probably forced by the ring substituents into similar conformations which are readily recognised by the receptors. These steric effects of the methyl groups in AMPA and 4-methyl-homoibotenic acid and the similarity of the proposed resulting conformations are shown in Fig. 2. The depicted orientation of the side chain of AMPA is supported by X-ray crystallographic studies²⁶, and further studies along these lines may elucidate the 'receptor-active conformation(s)' of Glu.

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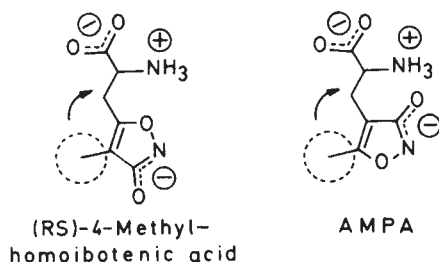


Fig. 2 The structures of 4-methyl-homoibotenic acid and AMPA at physiological pH and their proposed conformations determined by the steric influences of the methyl groups.

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Glucagon and glicentin immunoreactivity are topologically segregated in the α granule of the human pancreatic A cell

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Glucagon is a 100-amino acid polypeptide purified from the porcine intestine¹ and containing the immunodeterminants of glucagon². We have previously reported³ the presence of glicentin immunoreactivity in the glucagon-containing, specific secretory granules (α granules) of the pancreatic and gastric A cell. With an improved immunocytochemical probe, the protein A-gold (pAg) technique⁴, we are now able to show that glucagon and glicentin-like material are topologically segregated in the α granule of the human pancreatic A cell.

Surgical specimens of the pancreatic tail were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, dehydrated and embedded in Epon 812. Thin sections of the embedded blocks selected for the presence of islets of Langerhans were collected on Parlodion-coated nickel grids and processed for the pAg technique according to the method of Roth *et al.*⁴. The thin sections attached to the grids were incubated for 2 h at room temperature on a drop of anti-glucagon or anti-glicentin serum, washed carefully with phosphate-buffered saline (PBS) and further incubated for 1 h at room temperature with the protein A-gold complex. After a rinse in PBS and distilled water, incubated sections were double stained with 1% aqueous uranyl acetate and 1% lead citrate. The anti-glucagon serum was the 15K antiserum; 15K is a C-terminal anti-glucagon serum which does not react with extracts containing gut GLI's (R. H. Unger, personal communication). 15K was used in a 1:20 dilution. The specific anti-glicentin serum (R64) has already been characterised³, and was used at 1:400 dilution. Protein A-gold solution prepared according to the method of Roth *et al.*⁴ was used at a 1:15 dilution. In some experiments the same thin section was successively incubated with 15K and R64 antisera before exposure to the pAg solution. Thin sections were examined in a Philips EM 301 electron microscope.

The specific secretory granules of the human pancreatic A cell following glutaraldehyde and osmium tetroxide fixation and uranyl and lead staining appear as roundish, membrane-limited vesicles with a non-homogeneous content. Characteristically, the content is formed by a round, electron-dense core surrounded by a mantle of less electron-dense material⁵. This typical morphology is preserved by the glutaraldehyde fixation

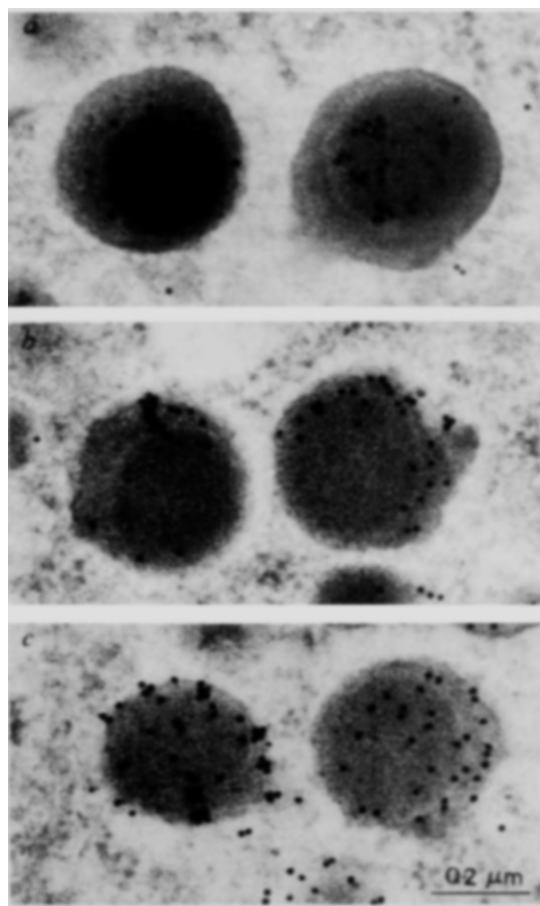


Fig. 1 Three different thin sections showing two human α granules (in near-equatorial section) stained with the pAg technique using 15K anti-glucagon (a), R64 anti-glicentin (b) and anti-glucagon followed by anti-glicentin (c) sera, respectively. All sections were contrasted with uranyl acetate and lead citrate. With the anti-glucagon serum (a), the gold particles revealing the site of antiserum binding on glucagon antigens are situated mostly over the roundish, slightly eccentric, electron-dense core of the α granule. The anti-glicentin serum (b) binds gold particles almost exclusively over the paler, peripheral mantle of the granule surrounding the dense core. The third granule profile appearing at the lower margin of b represents a tangential section through the granule core and shows little gold binding. The three gold particles to the right are interpreted as mantle binding; mantle also appears blurred due to tangential sectioning. The successive incubation with anti-glucagon and anti-glicentin sera (c) shows a homogeneous binding of gold particles over the entire surface of the granule. The deposit of gold particles at the lower margin of c represents the staining of a tangentially cut (and thus not readily discernible) α granule mantle (compare with b). a–c, $\times 64,000$.

alone used for the pAg technique. In sections incubated with the C-terminal anti-glucagon serum, the gold particles revealing the glucagon antigenic sites seemed to be mostly restricted to the dense core of the α granule (Fig. 1a). With the anti-glicentin serum, gold particles were seen mostly on the peripheral mantle (Fig. 1b). A quantitative evaluation of the labelling induced by each antiserum on 100 granules showing both the dense core and the mantle in each condition showed approximately 77% of gold particles located over the core in glucagon-immunostained sections and 77% of the particles over the mantle in glicentin-immunostained sections. In sections incubated successively with both specific antisera, approximately equal numbers of gold particles were associated with the dense core and with the mantle of the 100 granules evaluated in this condition (Fig. 1c). This labelling pattern was consistently obtained in several experiments with different sections and different blocks of tissue. Control experiments omitting the antiserum step or

carried out with an antiserum absorbed with its corresponding antigen before application of the pAg solution showed only occasional gold particles over some α granules.

Although the presence of glucagon and glicentin immunoreactive material was demonstrated previously in the secretory granules of the A cell by the peroxidase-anti-peroxidase technique of Sternberger⁶ using anti-glucagon and anti-glicentin sera³, a detailed analysis of the localisation of these antigens was not possible due to the masking effect of the dense, irregular deposits of peroxidase reaction product. With the much improved resolution of the pAg technique, glucagon and glicentin immunoreactivity seem not to be mixed in the secretory granule, at least as judged by morphological criteria. In favour of the specificity of mantle labelling following application of anti-glicentin serum is the former cytochemical demonstration that Grimelius' silver preferentially stains the mantle (and not the core) of the α granule⁷, as well as the entire secretory granule content in the L cell⁸, the specific glicentin-producing cell of the intestine³. Although the mechanism(s) by which glucagon and glicentin immunoreactive material are packed and maintained separately in the α granule is unknown, the apparent segregation of these two polypeptides offers a reasonable explanation

of the previously puzzling inhomogeneity of the α granule content. That the dual granule content is co-secreted during α granule release is indicated by recent evidence showing efflux of glicentin-like immunoreactivity by the perfused porcine pancreas (J. J. Holst, S. Lindkaer and A. J. Moody, unpublished data).

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Metastatic potential correlates with enzymatic degradation of basement membrane collagen

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Tumour cells traverse epithelial and endothelial basement membranes during the successive stages of the metastatic process. At the transition from *in situ* to invasive carcinoma, local dissolution of the basement membrane is observed microscopically^{1,2}, and coincides with tumour cell invasion of the underlying stroma. Tumour cells further traverse the endothelial basement membrane during entry into and egress from blood vessels³⁻⁵. Electron microscopic studies have shown local dissolution of basement membrane at its area of contact with invading tumour cells, suggesting an enzymatic mechanism^{3,6,7}. Basement membranes are resilient structures which present a mechanical barrier to invasion⁸. Type IV collagen is a major structural protein of basement membranes and is chemically and genetically distinct from stroma collagen types I and III and cartilage collagen type II^{9,10}. Previously characterised animal collagenases which cleave collagen types I, II and III fail to degrade type IV collagen^{11,12}. We have recently purified about 1,000-fold and characterised a neutral protease activity preferential for type IV collagen from metastatic tumour cells and shown that it (1) produces specific degradation products, (2) has a molecular weight of 65,000, (3) is not plasmin or a cathepsin, by pH and inhibitor studies, and (4) does not significantly degrade other collagens or fibronectin^{12,13}. Here we extend the relevance of this finding by quantitating the ability of several murine tumour cell lines of known metastatic potential to degrade type IV collagen. The cell lines with the highest incidence of spontaneous metastasis exhibit the greatest level of type IV collagen-degrading activity in two different assays using either living cells or media obtained from cell cultures.

The metastatic murine tumour cell lines used here were variants of the B16 melanoma and the T241 sarcoma. Cell lines F₁ and F₁₀ selected from the B16 melanoma exhibit a 10-fold difference in metastatic efficiency^{14,15} (number of lung colonies

per cell following tail vein injection). Line B16-BL6 is a variant with increased invasive capacity *in vitro* and *in vivo* selected from F₁₀ by injecting these tumour cells *in vivo* into the mouse bladder via the vas deferens. The bladder was then excised and cultured in 0.5% soft agar. Colonies in the agar that arose from cells which had invaded the bladder wall were collected and plated in Dulbecco's modified Eagle's medium and 10% fetal calf serum (FCS). When sufficient numbers of cells had grown in culture, they were cycled back through the bladder a total of six times (G. Poste, I. Hart and I. J. Fidler, in preparation). Line

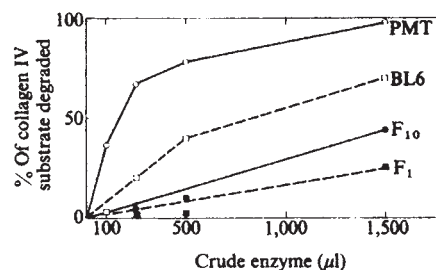


Fig. 1 Basement membrane collagen-degrading activity in culture media from metastatic cell lines. Tumour cells and control cells were grown to log phase in T75 tissue culture flasks. Human polymorphonuclear leukocytes were isolated from the blood of normal volunteers by the Ficoll-Hypaque method, followed by dextran sedimentation and hypotonic lysis to remove erythrocytes. The leukocytes were suspended in Hank's balanced salt solution at a concentration of 4×10^6 cells per ml, and were incubated at 37 °C. Thirty minutes after the addition of FMLP chemotactic peptide, the cells were separated from the medium by centrifugation. The supernatant was assayed for type IV collagen degrading activity. As previously shown¹², 25-50% saturation of ammonium sulphate precipitates virtually all the enzyme activity. The precipitate was dissolved in and dialysed against the reaction buffer (0.05 M Tris-HCl, 0.2 mM NaCl, 10 mM CaCl₂, pH 7.6) in such a volume that it became 20 ml per 10^6 viable cells to assess direct comparison between cell number in original cultures. The reaction was preceded by trypsin activation of the enzyme by 0.25 volumes of trypsin ($10 \mu\text{g ml}^{-1}$) at 37 °C for 4 min followed by trypsin inhibition by 0.25 volume of soybean trypsin inhibitor ($50 \mu\text{g ml}^{-1}$). The enzyme reaction was carried out with various volumes of the activated enzyme at 37 °C for 4 h in a final volume of 1.6 ml containing 50 μl (2,500 c.p.m.) of ¹⁴C-proline-labelled type IV collagen in the reaction buffer. The undigested substrate was precipitated with 200 μl of a solution of 10% trichloroacetic acid and 0.5% tannic acid using 20 μl of bovine serum albumin (1 mg ml⁻¹) as a carrier. After precipitation at 4 °C for 30 min the tubes were spun at 2,500 r.p.m. for 15 min and aliquots of the supernatant were counted in a liquid scintillation counter. Enzyme activity is expressed as radioactivity (c.p.m.) released from substrate per μl of activated enzyme. The relative activity of the cell lines computed from the linear portion of the dilution curve is shown in Table 2. Maximum activity (100% in Fig. 1) is the number of counts released by bacterial collagenase. Trypsin digestions were used to control for any nonspecific protease activity in the samples. Trypsin ($0.5 \mu\text{g ml}^{-1}$) digested 14.0% of the substrate.

Table 1 Degradation of ^{14}C -proline-labelled basement membrane collagen by living cells

Cell type	Type IV collagen degradation (c.p.m. per 10^5 cells)	Incidence of spontaneous lung metastases*	Median (range) no. of pulmonary metastases following tail vein injection (5×10^4 cells) ($n = 10$)
Adult mouse fibroblasts	No detectable activity	None	None
Tumorigenic but non-metastatic transformed fibroblasts	No detectable activity	None	None
Cornea fibroblasts	No detectable activity	None	None
F ₁	35.0 ± 11.0	None	13 (2–48)
F ₁₀	82.1 ± 9.0	30% (within 4 weeks)	500 (170–500)
BL6	152.9 ± 15.5	80% (within 4 weeks)	163 (43–264)
PMT	329.7 ± 38.0	100% (within 2 weeks)	172 (82–310)

Costar tissue culture cluster wells were coated with ^{14}C -proline-labelled type IV collagen by applying 0.2 ml (2.5×10^3 c.p.m.) of a solution of the collagen in 0.01 M acetic acid to each well followed by evaporation. Cells in log phase of growth were collected by 0.1% EDTA with 80% viability and first washed with complete medium (RPMI-1640 + 10% FCS) and then with serum-free medium. Cell viability was judged by trypan blue exclusion. From 1×10^3 to 3×10^6 viable cells were inoculated into coated wells in a volume of 2 ml serum-free medium. After 10 h 1 ml of medium from each well was collected, centrifuged at 2,000 r.p.m. for 10 min at 4°C and soluble radioactive digestion products were counted in a scintillation counter. Values shown are based on cell dose-dependent determinations and are related to the number of viable cells. The enzymatic activity is compared with the ability of these cells to form metastases. Both spontaneous lung metastases (derived from the transplanted tumour) and artificially induced lung metastases were studied. Note that the relative activity of the cell lines parallels the spontaneous metastatic properties more closely than the intravenous metastatic assay. Culture medium alone released 45 ± 20 ^{14}C c.p.m. per well or approximately 2.0% of applied counts. Purified bacterial collagenase (10 units) released $2,200 \pm 50$ c.p.m. or 90% of applied counts. Type IV collagen degradation was linear with time over the first 20 h. If soluble counts in an assay did not exceed counts released by culture media alone, the result was scored as no detectable activity.

* After intramuscular injection of 25,000 cells and amputation of the primary tumour ($n = 20$).

PMT was selected from a pulmonary metastasis of the T241 sarcoma as described previously¹⁶. Cell strains ACT and TACT are normal and spontaneously transformed adult mouse connective tissue cells, respectively, obtained by a method first described by Boone¹⁷. TACT cells are tumorigenic in 100% of mice but are non-metastatic.

Our first type of assay for type IV collagen-degradation activity consisted of inoculating living cells into tissue culture cluster wells coated with labelled substrate. The ^{14}C -proline-labelled type IV collagen used as substrate in our assays was prepared biosynthetically in organ cultures of a basement membrane-producing tumour (transplantable EHS sarcoma) from C57BL/6J mice. Methods for purification of the substrate have been described in detail previously^{18,19}. Cells in log growth phase were collected using 0.1% EDTA, and known numbers of viable cells were plated into each well. Degradation of the collagen layer resulted in the release of soluble counts into the medium which, for cells exhibiting activity, was verified in multiple assays to be proportional to the cell number. As shown in Table 1, the tumour cell lines exhibiting the highest activity *in vitro* also exhibited the highest propensity for spontaneous metastasis. However, this assay did not distinguish cell release of collagenase activity from cell phagocytosis of the substrate and intracellular digestion. We therefore searched for type IV collagenolytic activity secreted by these cells. Media from log phase cultures of these cell lines were collected and enzyme activity was precipitated from the media with 25–50% saturation of ammonium sulphate. The enzyme digestion consisted of

native ^{14}C -proline-labelled type IV collagen in solution incubated with trypsin-activated samples at pH 7.6 for 4 h. The undigested substrate was precipitated with 2.0% trichloroacetic acid and 0.08% tannic acid, and the digestion products were counted for radioactivity in the supernatant. This rapid assay for type IV collagenolytic activity, as elsewhere described^{12,13,20}, is highly reproducible. As shown in Fig. 1, the rate of the type IV collagenolytic activity was linear until about 60% of the substrate had been degraded. When the specific activity for each cell line was calculated from the linear portion of the concentration curves, the cell lines with the highest rate of spontaneous metastases *in vivo* exhibited the highest activity (Table 2).

The metastatic process is complex, involving many successive anatomic barriers which must be traversed by the malignant cell and many points where tumour cells can interact with host defences⁴. The ability to degrade basement membranes enzymatically may augment the aggressive behaviour of some metastatic cells. These cells may also differ from non-metastatic cells in other properties such as cell-surface proteins²¹. Furthermore, such an enzymatic activity is probably present in non-malignant cells in conditions where basement membrane is turned over¹². The metastatic tumour cell may exhibit a quantitative difference from normal cells in the synthesis of this enzyme and therefore measurement of its activity might serve as a biochemical marker *in vitro* for the metastatic potential of malignant cells.

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Table 2 Basement membrane collagen-degrading activity secreted by cultured cells

Cell type	Type IV collagenolytic activity (c.p.m. per 2×10^5 cells)*
1. Mouse fibroblasts	None detected
2. Bovine aorta smooth muscle cells	None detected
3. Bovine cornea fibroblasts	None detected
4. TACT cells†	None detected
5. F ₁	398 ± 36
6. F ₁₀	714 ± 62
7. BL6	$2,274 \pm 136$
8. PMT	$8,230 \pm 214$
9. Bovine vein endothelium	56 ± 27
10. Human leukocytes‡	880 ± 90
11. Human leukocytes + chemotactic peptide‡	$2,640 \pm 240$
12. MCF-7 Human breast carcinoma cells	$1,210 \pm 180$

None detected: digestion in the assay was less than trypsin or media controls.

* Lines 1–8, $n = 10$; lines 9–11, $n = 5$. Mean $\pm$ s.d.

† Tumorigenic but non-metastatic spontaneously transformed mouse connective tissue cells.

‡ Experiments in collaboration with Verena Muller and Eliot Schiffman, National Institute of Dental Research, NIH. (V. Muller *et al.*, in preparation.)

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Terminal differentiation in human promyelocytic leukaemic cells in the absence of DNA synthesis

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Some phorbol diesters, the most potent of which is 12-O-tetradecanoylphorbol-13-acetate (TPA), exert two different effects on the differentiation of leukaemic cells *in vitro*: they reversibly inhibit the differentiation in Friend mouse erythroleukaemia cells^{1,2} and mouse myeloid leukaemia M1 cells³, and they induce differentiation in other mouse erythroleukaemia cells⁴, mouse myeloid leukaemia cells⁵, human promyelocytic leukaemia cells⁵⁻⁹ and cells of patients with myeloid and myelomonocytic leukaemia¹⁰. The effect on human leukaemic cells is both irreversible and independent of the continuous presence of the drug⁷. We report here that TPA-induced differentiation of human promyelocytic leukaemia cells (HL60) need not be preceded by a round of DNA synthesis, and that the majority of TPA-treated cells accumulate in the G1 phase of the cell cycle.

We and others have shown previously that TPA-treated HL60 cells 'terminally' differentiate into macrophage-like cells rather than mature myeloid cells^{5,7-9}. The evidence was based on either the appearance of markers in TPA-treated HL60 cells that are specific to the monocytic/macrophage lineage or the disappearance of myeloid markers. One monocytic marker is α -naphthyl acetate esterase (NAE), an enzyme which can be detected in the human bone marrow only in megakaryocytes or in monocytes¹¹; another is the presence of two acid phosphatase isozymes, 3a and 3b (ref. 12), which appear 3 d after TPA treatment⁹. Some myeloid markers, like myeloperoxidase and chloracetate esterase (CAE), are markedly reduced following TPA treatment of HL60 cells⁷. Markers common to both monocytic and myeloid cells, like phagocytosis, immunoreactivity, Fc receptors for immunoglobulin G, C3 receptors and lysozyme⁵⁻⁸ are also present after TPA treatment of HL60 cells. Although of little use in defining TPA-specific

differentiation, these markers are general indicators of differentiation. The present studies were designed to establish the relationship between the appearance of these differentiation markers and the cell cycle of TPA-treated HL60 cells. Four differentiation markers were used—NAE, CAE, acid phosphatase and its isozymes, and phagocytosis.

Figure 1 indicates that freshly plated, untreated HL60 cells, exponentially growing in culture, do not contain a sizeable fraction of cells in a resting state: continuous labelling with ³H-thymidine (TdR) for 48 h shows that 99% of the cells go through the S phase of the cell cycle at least once. Labelling of the cells for 1 h at the beginning of the experiment shows that about 48% of the exponentially growing cell population is at that time in S.

However, if TPA and ³H-TdR are added simultaneously to freshly plated HL60 cells, only a fraction of the cell population is labelled, even if the cells are exposed to the isotope for 72 h or more. This labelled fraction (52–55%) is only slightly in excess of that obtained by 1-h pulse labelling, which suggests that only a small fraction of the cells (~4–7%) which were not in S at the time of TPA addition are able to enter S after TPA treatment. Instead, most of the cells that were not in S (but in G2, M or G1) will never enter S after TPA treatment. The possibility that the lack of ³H-TdR incorporation in this subpopulation is a result of decreased TdR uptake, associated with normal cell proliferation, is ruled out as only a small increase in cell number is observed shortly after TPA treatment, and there is no increase at all after 12 h (ref. 7). As shown in Table 1, essentially all the TPA-treated cells became intensely positive for NAE (72 h after treatment), and CAE activity disappeared. Acid phosphatase was positive in almost all TPA-treated cells, and analysis of acid phosphatase isozymes by acrylamide gel electrophoresis confirmed that the monocytic-specific bands, 3a and 3b (ref. 9), were induced (not shown). Because about 48% of the TPA-treated cells never entered the S phase, it is clear that acid phosphatase and NAE could be expressed in TPA-treated cells

Table 1 Percentage of HL60 cells expressing differentiation markers

Experimental conditions	NAE	CAE	AcPh	Phagocytosis	
				³ H-labelled	Unlabelled
Untreated	1	80	9	3	2
TPA-treated	99	4	88	42	40

Cells were collected 72 h after TPA treatment and assayed for α -naphthyl acetate esterase (NAE), naphthol-AS-D chloroacetate esterase (CAE), acid phosphatase (AcPh) and phagocytic ability. A minimum of 500 cells was scored by two independent observers. Values represent averages of duplicate experiments; phagocytosis was scored in conjunction with autoradiography of ³H-TdR-labelled cells to determine whether cells bypassing the S phase developed phagocytic activity. Assay procedures are described in refs 7, 9 and 10.

without the cells undergoing a new round of DNA synthesis. We can also rule out the possibility that these marker enzymes are expressed constitutively in G1 because at any given time, 40–50% of the untreated cells were in G1 (see Fig. 2) but only 1% were positive for NAE and 10% were positive for acid phosphatase (Table 1).

Phagocytic activity was assayed in TPA-treated cells labelled continuously with ³H-TdR for 3 days. Following autoradiography, positive and negative cells were scored for both acid-insoluble radioactivity in the nucleus and the presence of latex beads in the cytoplasm (Table 1). Both the cell populations that went through S and those that did not develop phagocytic capability.

From these experiments, we concluded that transition through the S phase was not required for differentiation of TPA-treated HL60 cells into macrophage-like cells. It was still not clear, however, whether TPA treatment caused cells to stop in different phases of the cell cycle (an indication of a toxic effect on the cells) or in one particular phase. To answer this question, we pulse labelled (1 h) HL60 cells with ³H-TdR at different

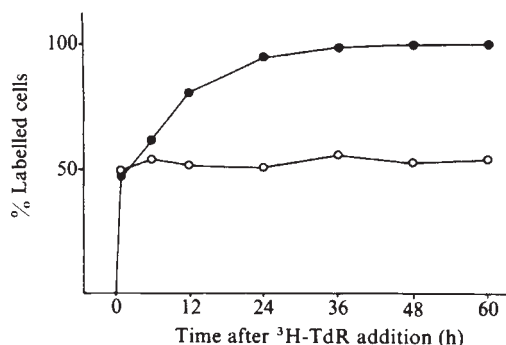


Fig. 1 HL60 cells^{16,17} were grown and induced to differentiate with 1.6×10^{-8} M TPA, as previously described⁷. TPA-treated and untreated cells were continuously labelled with ³H-TdR ($0.1 \mu\text{Ci ml}^{-1}$; 6.7 Ci mmol^{-1}), collected by cytocentrifugation at the times indicated in the text and the figure, and processed for autoradiography¹⁵. Percentage of labelled cells are plotted against hours after addition of ³H-TdR. The cumulative percentage of labelled cells was determined at the time indicated. TPA and ³H-TdR were added to the treated cells simultaneously. ●, Untreated cells; ○, TPA-treated cells. Values represent the averages of two different experiments.

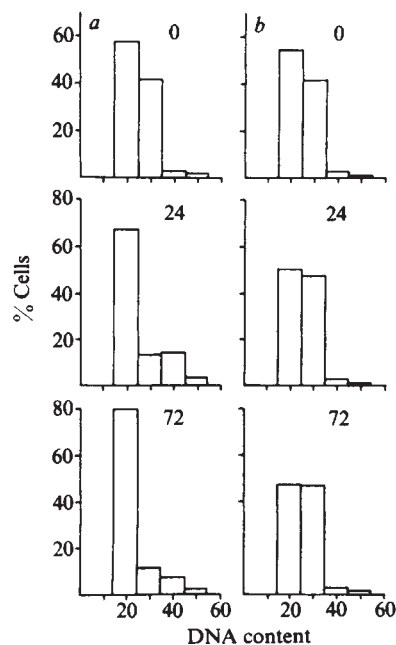


Fig. 2 Percentage of cells plotted against DNA content in arbitrary units. *a* Represents the distribution of DNA content in TPA-treated HL60 cells, and *b*, the distribution of DNA content in untreated HL60 cells. Numbers in the upper right corners indicate hours after plating the cells with or without TPA. HL60 cells have a modal chromosome number of 44 (refs 16, 17). The DNA content of a minimum of 200 cells per time point was determined with a Vickers M85 scanning microdensitometer after Feulgen staining. A standard was obtained by blocking the cells in S phase with fluorodeoxyuridine.

times after the addition of TPA and determined the percentage of cells that incorporated ^3H -TdR into their DNA (Fig. 3). This percentage was found to decrease progressively after TPA treatment. As this result could be interpreted as either a decreasing number of cells in S and normal TdR uptake, or a constant number of cells in S and decreasing TdR uptake, we determined the DNA content in each cell at different times after TPA treatment. The results shown in Fig. 2 indicate that the population of cells in S did, in fact, progressively decrease. The DNA distribution of untreated HL60 cells at different times after seeding was relatively constant: approximately 52% of the cells had a G1-level DNA content, 8% had a G2-level content, and 40% an S-level intermediate content. Following TPA treatment, there is a progressive decrease in the fraction of cells

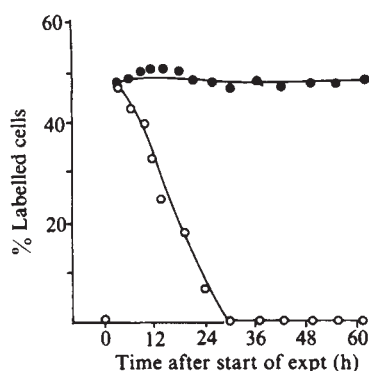


Fig. 3 Percentage of labelled cells plotted against hours after the beginning of the experiment. HL60 cells, either untreated (●) or treated (○) with 1.6×10^{-8} M TPA were pulse labelled for 1 h with ^3H -TdR ($0.1 \mu\text{Ci ml}^{-1}$) at different times after the beginning of the experiment. Values represent the averages of two different experiments.

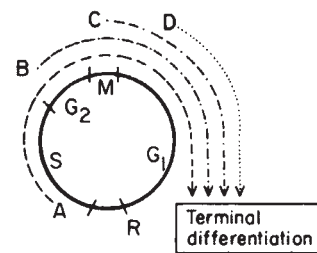


Fig. 4 Cell cycle model for TPA-induced differentiation of HL60 cells. R: point late in G1 beyond which TPA-treated cells may still proceed through one cell cycle. A, B, C, D, Fate of cells in S, G2, M and G1, respectively, at the time of TPA treatment.

with an S-phase DNA content, and an increase in the fraction of cells with a G1 DNA content. Eighty per cent of the cells were found to be G1 72 h after TPA treatment.

These results indicate that TPA-treated HL60 cells accumulate in G1 rather than stopping at various phases of the cell cycle. We have shown previously that the arrested cell cannot be restimulated to proliferate by changing the medium or by removing the TPA⁷. Rather, the cells remain quiescent until their death, 4 to 6 days after the addition of TPA.

The data presented here suggest the model of the TPA effect on the cycle of HL60 cells that is shown in Fig. 4. There is a stage, R, late in G1, after which TPA-treated cells will proceed through S, complete the cycle, stop in G1 and differentiate. Cells in G2, M or early-to-middle G1 at the time of TPA treatment will stop in G1 and differentiate without requiring new DNA synthesis.

The process of TPA-induced differentiation of HL60 cells into macrophage-like cells is rapid and lacks the multiple cell divisions normally associated with the transition from a promyelocytic to a more differentiated myeloid cell. Thus, TPA-induced differentiation differs from known *in vivo* and *in vitro* models of erythroid and myeloid differentiation. However, little is known about monocytic differentiation. It is generally accepted that monocytes and myeloid cells have a common progenitor¹³, and there is evidence for the extremely rapid *in vivo* formation of monocytes from precursors in the mouse¹⁴. Therefore, it is possible that TPA-induced differentiation *in vitro* may not be totally anomalous, but could mimic some *in vivo* process. Because TPA can force myeloid cells in every phase of the cell cycle to become irreversibly quiescent, this drug and others with a similar effect, such as mezerein⁸, may be useful as anti-leukaemic agents in experimental models^{5,6,8}.

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Phorbol ester-mediated stimulation of the synthesis of mouse mammary tumour virus

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12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is a potent tumour promoter in the two-stage mouse skin carcinogenesis model^{1,2}. Recently, TPA has been shown to enhance the transformation of cultured cells by two DNA tumour viruses—adenovirus³ and Epstein-Barr virus⁴. It is not known whether TPA also augments the transformation of cells by RNA tumour viruses. In Rous sarcoma virus (type C) transformed fibroblasts, the levels of plasminogen activator are elevated by TPA^{5,6}, a phenomenon also associated with malignant transformation^{7,8}. In addition, TPA and other promoters induce the synthesis of some herpesviruses in persistently infected cells^{9,10}. I have investigated the effect of TPA on the synthesis of mouse mammary tumour virus (MMTV), type B RNA tumour virus, and find that TPA, in nanogramme quantities, causes a 10- to 20-fold stimulation of MMTV production by persistently infected mouse mammary tumour cells. This stimulation is specific for TPA; the non-promoter, phorbol, does not stimulate MMTV production.

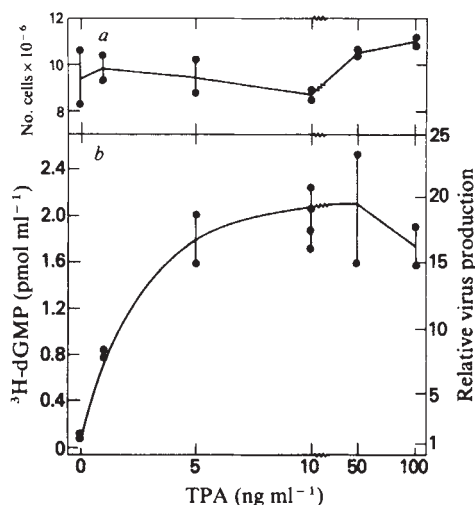


Fig. 1 Effects of TPA on cell growth (a) and virus production (b) by mouse mammary tumour cells grown in the absence of dexamethasone. Duplicate monolayer cultures of Mm5mt/c₁ cells were treated with TPA for 48 h. Twenty-four hours before collecting medium, cultures were given fresh medium with or without TPA. Samples of medium (18–20 ml per culture) were clarified and centrifuged at 25,000 r.p.m. for 90 min at 4 °C in a Beckman SW 27.1 rotor. The pellet was suspended in 0.01 M Tris-HCl (pH 7.8)/0.1 M NaCl/1 mM EDTA (TNE), layered on a column of 20% glycerol in TNE, and centrifuged at 35,000 r.p.m. for 60 min at 4 °C in a Beckman SW 50.1 rotor. The pellet was dissolved in 100–200 µl of 0.01 M Tris-HCl (pH 7.8)–0.01 M NaCl and 20–40 µl aliquots were used for virus estimation by particle-associated RNA-directed DNA polymerase assay. The polymerase assays utilizing poly(C): oligo(dG) as a template: primer and magnesium ion as a cofactor were performed as described before^{13,14}. The data in (a) show the number of cells per culture and in (b) show the amount of ³H-labelled dGMP (3,500 c.p.m. pmol⁻¹) incorporated into acid insoluble material by purified particle preparation corresponding to 1 ml of collected medium.

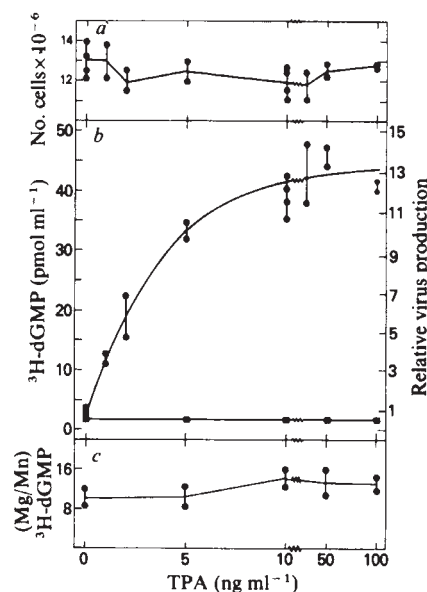


Fig. 2 Effect of TPA (○) and phorbol (●) on cell growth (a) and virus production (b) by mouse mammary tumour cells grown in the presence of dexamethasone (2 µg ml⁻¹). Experimental details as for Fig. 1, except the specific activity of ³H-dGTP used for polymerase assays was 700 c.p.m. pmol⁻¹. The data in (c) show the ratio of magnesium (20 mM) over manganese (1 mM) cofactored DNA polymerase activity of purified particle preparations.

MMTV producing C3H mouse mammary tumour derived cells, designated Mm5mt/c₁ (refs 11, 12), were treated with TPA for 48 h in the presence or absence of dexamethasone. Figure 1 shows the effect of TPA on virus production when the cells were grown in medium without dexamethasone. Clearly, TPA markedly enhanced virus production without significantly affecting cell growth. The effect was dose-dependent: at 1 ng ml⁻¹, the virus production was stimulated by about 8-fold; at 10 ng ml⁻¹, the stimulation was nearly 20-fold. The increased virus production could not be ascribed to the induction of endogenous type C virus(es). The particle-associated DNA polymerase of MMTV prefers magnesium ions as cofactor while that of type C viruses prefers manganese ions^{13,14}. Thus the ratio of polymerase activity in the presence of magnesium and manganese ions is characteristic of the virus type. In our case, TPA did not change this ratio in ways suggesting the induction of type C virus(es) (see Fig. 2c). As dexamethasone also enhances the synthesis of MMTV^{12,15,16}, we determined whether TPA would act synergistically with dexamethasone. As shown in Fig. 2, TPA stimulated virus production even when the cells were grown in the presence of dexamethasone (2 µg ml⁻¹). Again the effect was dose-dependent. The maximum stimulatory effect of 10- to 12-fold was obtained at a TPA concentration of 10 to 20 ng ml⁻¹. As dexamethasone alone stimulated virus production 10- to 20-fold the combined effect of dexamethasone and TPA apparently resulted in 100- to 200-fold enhancement of virus production. This effect was specific for TPA; the non-tumour promoter, phorbol, failed to stimulate virus production up to a concentration of 100 ng ml⁻¹ (Fig. 2).

The effect of TPA on the steady state level of intracellular viral RNA was also examined. This was done by hybridising cellular RNA with representative MMTV complementary DNA (cDNA). The cDNA was prepared by transcribing 30–35S viral RNA with avian myeloblastosis virus reverse transcriptase in the presence of calf thymus DNA oligomers¹⁷. The cDNA contained a uniform representation of 75–80% of viral sequences; other sequences were less frequently represented

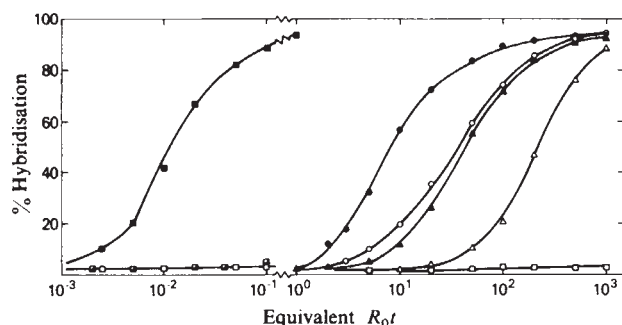


Fig. 3 Effect of TPA on the steady state level of intracellular viral RNA in mouse mammary tumour cells grown in the presence and absence of dexamethasone. Cellular RNA was isolated by phenol extraction and DNase digestion. A solution of RNA ($0.5\text{--}1\text{ mg ml}^{-1}$) and cDNA ($20,000\text{ c.p.m.}$ or 1 ng ml^{-1}) in $0.3\text{ M NaCl}/0.03\text{ M Na citrate } 2\text{ mM EDTA } 0.02\%$ sodium lauryl sarcosinate ($\text{pH } 7$) was incubated at 65°C . Aliquots were withdrawn at intervals and hybrids were scored by S_1 nuclease digestion. The R_{0t} [concentration of RNA (mol l^{-1}) $\times$ time (s)] values have been reduced to standard conditions of 0.18 M NaCl and 60°C ¹⁸. The curves represent cellular RNA from untreated cultures (Δ), cellular RNA from TPA-treated (20 ng ml^{-1}) cultures ($\blacktriangle$), cellular RNA from dexamethasone-treated ($2\text{ }\mu\text{g ml}^{-1}$) culture ($\circ$), cellular RNA from cultures treated with dexamethasone ($2\text{ }\mu\text{g ml}^{-1}$) plus TPA (20 ng ml^{-1}) ($\bullet$), MMTV RNA ($\blacksquare$), murine leukaemia virus (AKR) RNA ($\square$), and yeast RNA ($\square$).

(data not shown). The MMTV cDNA did not hybridise with murine leukaemia virus (AKR) RNA (Fig. 3). Conversely, the cDNA of murine leukaemia virus (AKR) did not hybridise with MMTV RNA. Thus MMTV cDNA contained only MMTV specific sequences. The R_{0t} analyses revealed that TPA (20 ng ml^{-1}) increased the concentration of intracellular MMTV specific RNA 5- to 7-fold relative to control cultures (Fig. 3). Dexamethasone ($2\text{ }\mu\text{g ml}^{-1}$) alone caused a 6- to 7-fold increment in the concentration of intracellular viral RNA and this was increased a further 5-fold by TPA (20 ng ml^{-1}). These results suggest that TPA causes an increase in the transcription of viral RNA accompanied by a stimulation of assembly and release of virus particles. Note that the magnitude of TPA-caused enhancement of intracellular viral RNA does not strictly correspond to its effect on extracellular virus production. At a TPA concentration of 20 ng ml^{-1} , the level of intracellular viral RNA was increased 5- to 7-fold while extracellular virus production was enhanced 15- to 20-fold. Further, it is not yet known whether TPA directly affects some activity specific for virus synthesis or whether its effect is mediated through some cellular function. The effect of TPA seems to be specific for the synthesis of MMTV; TPA, up to 100 ng ml^{-1} , did not enhance the production of type C virus(es) by late passage Mm5mt/c₁ cells. The biological significance of these results in terms of mammary tumour development in whole animals remains to be ascertained.

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Sister chromatid exchange in cells metabolically coupled to Bloom's syndrome cells

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Cells from persons with Bloom's syndrome (BS) show many more sister chromatid exchanges (SCEs) than cells from normal individuals or those with any other genetic disease studied so far¹. Several other abnormalities demonstrable in cell culture (reviewed in ref. 2) suggest that the molecular defect in BS is related to DNA metabolism; for example, many chromosomal aberrations in untreated cells³, abnormally slow DNA chain growth^{4,5}, and increased sensitivity to treatment with ethyl methanesulphonate^{6,7}. BS cells respond as normal cells do in excision repair⁸⁻¹⁰, 'post-replication repair'¹¹, and repair of single-strand breaks¹². This apparent proficiency at repair prompted the suggestion^{13,14} that the various chromosome abnormalities result from an excessive accumulation spontaneously of lesions in the DNA on which a repair system(s) must act, thereby overloading the system(s). Such lesions might result, for example, from the action of a genetically defective enzyme involved in the semi-conservative replication of DNA, a defect leading to a quantitative imbalance in a chromosomal component, or a defect leading to the intrinsic production of a molecule with mutagenic properties. Experiments designed to test this last possibility have resulted in conflicting reports¹⁵⁻¹⁸. We report here results which do not support the hypothesis that BS cells release such a mutagenic molecule. SCE frequencies in non-BS cells were not affected when the cells were placed in contact-dependent metabolic coupling with BS cells. Also, when we repeated experiments¹⁵ in which medium 'conditioned' by BS fibroblasts was reported to increase the amount of SCE in normal blood lymphocytes, we were unable to duplicate those results.

Lesch-Nyhan syndrome fibroblasts, deficient in hypoxanthine guanine phosphoribosyltransferase (HGPRT⁻ cells), were co-cultivated with BS fibroblasts in hypoxanthine, aminopterin and thymidine (HAT)¹⁹ selective medium. In such conditions, the only HGPRT⁻ cells to proliferate are those metabolically coupled to BS cells²⁰, presumably due to the free exchange of nucleotides through gap junctions²¹. Pairs of cell lines used were opposite in sex—HGPRT⁻ male and BS female. After 3 d of co-cultivation, metaphase chromosome preparations were made. Chromosomes were stained with quinacrine and studied by fluorescence microscopy to determine the presence or absence in each metaphase of a Y chromosome. The chromosomes were then stained to give sister chromatid differentiation, and SCEs were counted. Both experimental and control co-cultivations were performed twice (Table 1, experiments 1 and 2); different lines of HGPRT⁻, BS and normal cells

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Table 1 Effect of co-cultivation on SCE frequencies in HGPRT⁻, BS and normal fibroblasts

Cell line(s) in culture		HGPRT ⁻ cells		SCE per cell BS cells		N cells	
Component A	Component B	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range
Experiment 1							
HGPRT ⁻ 1		8.7 $\pm$ 0.5	3-14 (30)				
HGPRT ⁻ 1*		9.3 $\pm$ 0.8	4-17 (24)				
	BS 1			60.0 $\pm$ 2.0	32-80 (30)		
	N 1					10.1 $\pm$ 0.8	3-20 (30)
HGPRT ⁻ 1	+BS 1	10.0 $\pm$ 0.7	5-18 (24)	58.8 $\pm$ 3.1	33-95 (19)		
HGPRT ⁻ 1	+N 1	9.6 $\pm$ 0.5	5-16 (37)			9.5 $\pm$ 0.9	5-16 (18)
Experiment 2							
HGPRT ⁻ 2		8.3 $\pm$ 0.6	4-16 (30)				
HGPRT ⁻ 2*		5.0 $\pm$ 0.4	1-9 (30)				
HGPRT ⁻ 2*		5.8 $\pm$ 0.5	0-14 (30)				
	BS 2			40.8 $\pm$ 1.4	21-56 (30)		
	N 2					8.6 $\pm$ 0.5	4-16 (29)
	N 2*					5.7 $\pm$ 0.4	2-10 (30)
HGPRT ⁻ 2	+BS 2	6.1 $\pm$ 0.4	1-14 (50)	43.7 $\pm$ 1.6	22-58 (29)		
HGPRT ⁻ 2	+N 2	5.8 $\pm$ 0.4	2-12 (26)			6.2 $\pm$ 0.4	1-12 (59)

The fibroblast lines used were actively growing, between the 9th and 22nd *in vitro* passages. Trypsin was used in passaging (1:2 splits) and collection. The HGPRT⁻ cell lines were GM-1662 (referred to as HGPRT⁻ 1) and GM-1362 (HGPRT⁻ 2) obtained from the Human Genetic Mutant Cell Repository at Camden. The BS cell lines were HG 916 (BS 1) and HG 369 (BS 2) derived in this laboratory from skin biopsies taken from patients identified in the Bloom's Syndrome Registry²⁶ as, respectively, 53(StAs) and 26(SaTi). The normal control fibroblast lines (referred to as N in these tables), also derived from skin biopsies taken here, were HG 883 (N 1) and HG 846 (N 2). All co-cultivations were initiated at 1:1 cell ratios. Medium was Eagle's minimal essential medium (MEM) with 10% fetal bovine serum (FBS), penicillin (50 U ml⁻¹), and streptomycin (50 µg ml⁻¹). All medium, except that used for cultures of HGPRT⁻ cells alone, was supplemented with hypoxanthine (2×10^{-2} M), aminopterin (0.8×10^{-4} M) and thymidine (3.2×10^{-3} M) (HAT medium). Cultures were gassed with a mixture of 10% CO₂ in air and stoppered. Initial cell seeding for all cultures was at 3.5×10^5 cells per 8-ounce glass flask. Twenty-four hours after seeding, cells were provided with fresh medium to which 10 µM BrdU had been added. Thereafter, cultures were incubated in the dark until metaphase preparations were made 48 h later by standard techniques. Metaphases from cultures containing two cell lines were stained with quinacrine and studied by fluorescence microscopy. The locations of all diploid metaphases with good morphology were recorded (cells with 43 or fewer chromosomes being rejected) and each metaphase was classified as to the presence or absence of a Y chromosome. Slides were then rinsed in demineralised water and dried overnight. On the following day, the metaphases were stained for sister chromatid differentiation with Giemsa stain, as described previously²⁷. Metaphases previously identified by fluorescence were relocated on the slides and studied for SCEs. SCEs were counted in metaphase cells which had gone through two rounds of DNA replication in the presence of BrdU and which showed good quality sister chromatid differential staining. Metaphases from cultures containing one cell line were stained for sister chromatid differentiation, and SCEs were counted in up to 30 consecutive cells meeting the criteria described above. SCE was determined for some cell lines several times during the course of the experiments, indicated by an asterisk. Numbers of cells analysed are shown in parentheses.

were used in the two sets of experiments. As Table 1 shows, HGPRT⁻ cells in metabolic coupling to BS cells for two cell cycles maintained their normal numbers of SCEs, and they had no effect on SCE in the BS cells.

To determine whether the experimental system had any effect on the amount of SCE present, both lines of HGPRT⁻ cells were co-cultivated in HAT medium with fibroblasts from normal females. The amount of SCE did not differ between co-cultivated and control HGPRT⁻ or normal female cells (Table 1). The HAT medium itself had no effect on SCE in either BS or normal fibroblasts (data not shown).

Published data¹⁵ indicate that medium conditioned by a line of BS fibroblasts increased SCE in phytohaemagglutinin (PHA)-stimulated blood lymphocytes from a normal individual. We repeated that experiment, using 'conditioned' medium from the same BS fibroblast line; also tested were media from two other BS lines and two lines of normal fibroblasts. Medium 'conditioned' by neither BS nor normal fibroblasts affected SCE in the blood lymphocytes of two normal individuals (Table 2).

Data from other laboratories¹⁶⁻¹⁸ indicate that the amount of SCE is reduced in BS fibroblasts in various conditions of co-cultivation, the size of the decrease depending on the initial proportions of cells used. While it is noteworthy that we observed no such effect, the altered metabolism of the non-coupled HGPRT⁻ cells in HAT medium may have precluded it. Thus, while the data presented here do not confirm the reports just cited¹⁶⁻¹⁸, further investigation, designed specifically to study that particular issue, will be required.

Bryant *et al.*²² reported experiments in which cell hybrids between diploid BS and diploid normal fibroblasts were produced; the hybrid cells had the low amount of SCE characteristic of the normal parental cells. With respect to the representation in their genomes of the BS gene, the hybrid cells are the equivalent of tetraploid cells occasionally found in cultures from BS heterozygotes, which have normal numbers of SCEs (our unpublished observations) as do diploid cells from BS heterozygotes^{1,23}. As such, therefore, these hybrids²² give no insight as to whether BS cells produce a factor that can increase SCE in normal cells.

In our experiments (Table 1) every HGPRT⁻ cell showing sister chromatid differential staining was coupled metabolically to a BS cell(s) for two cycles of DNA replication, ensuring exchange of small molecules between the cells²¹. The molecular size limit for exchange through mammalian gap junctions is approximately 900 daltons²⁴, a limit which encompasses essentially all mutagenic molecules currently recognised²⁵. We conclude that no such molecule was transferred from BS to HGPRT⁻ cells. Neither did our results support the idea that BS cells secrete a mutagen of greater than 900 daltons into the culture medium to be taken up by non-BS cells.

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Table 2 The effect on SCE in normal lymphocytes of medium 'conditioned' by BS fibroblasts

Source of medium	SCE in normal lymphocytes			
	Blood no. 1	Blood no. 2	Blood no. 1	Blood no. 2
	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range
Control	9.0 $\pm$ 0.6	2-18 (50)	8.4 $\pm$ 0.5	4-19 (50)
N 1	8.2 $\pm$ 1.0	3-12 (12)	7.9 $\pm$ 0.4	2-12 (50)
N 3	9.2 $\pm$ 0.5	4-25 (50)	8.0 $\pm$ 0.6	1-20 (50)
BS 1	9.4 $\pm$ 0.5	2-16 (50)	7.2 $\pm$ 0.5	2-16 (50)
BS 3	8.5 $\pm$ 0.4	2-16 (50)	7.3 $\pm$ 0.4	2-14 (50)
BS 4	10.4 $\pm$ 0.7	2-17 (28)	8.4 $\pm$ 0.5	4-20 (47)

PHA (Wellcome)-stimulated blood samples from two normal individuals were cultured in the dark in 5% CO₂ in Eagle's MEM supplemented with 15% FBS, 2 mM L-glutamine, penicillin (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and BrdU (25 µM). Forty hours after initiation, cultures handled in a dimly lit room were centrifuged at 1,000 r.p.m. for 8 min and half of the medium (5 ml) was replaced with either fibroblast-conditioned or control (not conditioned) medium prepared as described below. Cells were collected 24 h later for metaphase preparations by standard techniques. Staining for sister chromatid differentiation and criteria for cell selection were as for Table 1. Slides were coded, and SCEs were counted directly through the microscope ($\times 1,000$) in up to 50 consecutive metaphases. Fibroblast medium was as described for Table 4, experiment 2 of ref. 15 except that antibiotic concentrations were 100 U ml⁻¹ for penicillin and 100 µg ml⁻¹ for streptomycin. All fibroblast cultures were actively growing, between subculture passages 16 and 29 (1:2 splits). The final passage was by a 1:4 split, and 24 h later fibroblasts were rinsed with Earle's balanced salt solution and then provided with fresh medium. After further 4 d of incubation, medium was removed from nearly confluent cultures and centrifuged at 1,200 r.p.m. for 10 min to remove cellular debris. BrdU (25 µM) was added to both the conditioned media and the control medium, and these media were used immediately to replace half of the medium in the blood cultures. BS fibroblast lines used were HG 916 (BS 1), HG 1219 (BS 3) and GM-1492 (BS 4) from, respectively, 53(StAs), 81(MaGrou) and 44(ABRu). Control fibroblast lines were HG 883 (N 1) and HG 978 (N 3). Cell lines BS 1 and N 1 are the same as in Table 1. All cell lines were derived from skin biopsies taken in this laboratory, except GM-1492 which was obtained from the Human Genetic Mutant Cell Repository. Numbers of cells analysed are shown in parentheses.

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Induction of sister chromatid exchanges by BUdR is largely independent of the BUdR content of DNA

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The halogenated thymidine (dT) analogue, 5-bromodeoxyuridine (BUdR), has a variety of effects on mammalian cells, including toxicity, suppression of differentiation, and mutagenesis. Although it is generally assumed that the effects of BUdR are due primarily to its presence in DNA, results from our laboratory have raised doubts about such assumptions¹⁻⁴. We have shown, for example, that BUdR mutagenesis in mammalian cells is determined by the concentration of BUdR in the medium rather than in DNA³, and that mutagenesis can be suppressed by deoxycytidine (dC) without changing the amount of BUdR in DNA⁴. BUdR has also been shown to induce sister chromatid exchanges (SCEs) in mammalian cells⁵⁻⁷. Initial results suggested that the relationship between BUdR and SCEs might not be explained by a single factor⁵, and various correlations between BUdR and SCEs have been proposed^{8,9}. However, the results to date have been inconclusive, because the experiments did not resolve as independent variables the concentration of BUdR in the medium and the amount of BUdR incorporated into nuclear DNA. We have now carried out experiments to resolve these two factors; the results indicate that the major factor in determining the frequency of SCEs is the concentration of BUdR in the medium.

The cells used in these experiments were from a clone of Chinese hamster ovary (CHO) cells. The occurrence of SCEs was monitored by growing cells for two generations in the presence of BUdR and staining with the fluorescent bisbenzimidazole dye, 33258 Hoechst¹⁰, and Giemsa¹¹. In most experiments, the incorporation of BUdR into nuclear DNA and the induction of mutations by BUdR were measured in parallel cultures.

When CHO cells were grown in medium containing BUdR at concentrations ranging from 12 to 300 µM, the frequency of SCEs increased by over fourfold and the level of BUdR incorporation into DNA (BUdR substitution) also increased (Fig. 1a). To evaluate the role of the increased BUdR concentration *per se*, cells were grown in medium (FBT) containing 5-fluorodeoxyuridine (FUDR, an inhibitor of *de novo* dT biosynthesis) plus BUdR and dT at varying concentrations but at a fixed ratio. When CHO cells were grown in FBT medium containing 12-300 µM BUdR and with the BUdR:dT ratio fixed at 3:2, the frequency of SCEs increased by over threefold (Fig. 1a). However, because of the presence of FUDR and the fixed ratio of BUdR:dT, the level of BUdR substitution remained constant at approximately 60%. The increase in SCEs due to raising the BUdR concentration at a fixed level of BUdR substitution accounted for most of the increment in SCEs observed when the level of BUdR substitution was allowed to increase with the BUdR concentration. Similar data were obtained in several independent experiments. These results suggest that the major factor in determining the frequency of induction of SCEs is the concentration of BUdR in the medium rather than the amount of BUdR incorporated into DNA. The

Table 1 Induction of SCEs by BUdR

Medium additive (μ M)		BUdR substitution (%)	SCEs per cell	Mutant frequency
BUdRdT	dC			
Part 1: FUDR present				
12	24	38.8	7.5	<1
180	360	35.0	23.7	120
12	8	64.5	10.4	<1
180	120	59.1	22.6	214
12	2	85.0	12.9	<1
180	30	85.8	24.2	168
Part 2: FUDR present				
12	24	33.9	6.6	<1
12	8	64.5	9.3	<1
12	2	84.1	11.4	<1
12	—	95.1	14.4	NT
Part 3: FUDR absent				
12	—	75.8	8.7	<1
180	—	94.0	23.4	152
Part 4: FUDR present				
12	24	—	35.2	8.2
12	24	200	35.9	7.9
12	1	—	87.6	12.7
12	1	200	87.4	13.3

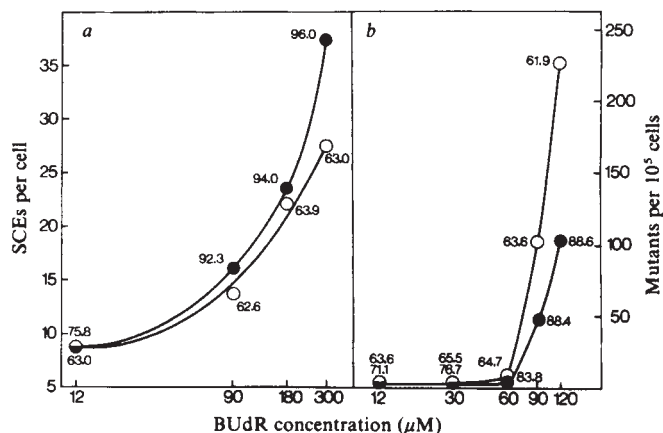
The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in either FBT medium (FUDR present) or E medium (FUDR absent) with the indicated concentrations of BUdR, dT and dC. NT, Not tested.

induction of mutations by BUdR in CHO cells was also determined by the BUdR concentration in the medium (Fig. 1b), in agreement with the results of our previous studies with Syrian hamster cells³. The parallel between the induction of SCEs and mutations by BUdR agrees with the relationship previously proposed for SCEs and mutations induced by other agents¹².

The results in Fig. 1a showed that increasing the BUdR concentration while holding the level of BUdR substitution constant at 60% resulted in a large increase in the frequency of SCEs. The ratio of BUdR:dT in FBT medium was varied, and similar tests were carried out with the level of BUdR substitution fixed as low as 35% or as high as 85%. As shown in Table 1, part 1, the relationship between BUdR concentration and SCEs was observed over the entire range of BUdR substitution levels tested.

Experiments were carried out to determine whether increasing the level of BUdR substitution had an effect of its own on the induction of SCEs. CHO cells were grown in FBT medium with a fixed concentration of BUdR (12 μM), and the level of BUdR substitution was increased by decreasing the amount of dT in the medium. As shown in Table 1, part 2, increasing the BUdR substitution in this manner from 34% to 95% had only a small (approximately twofold) effect on the frequency of SCEs, although this effect was reproducible in independent experiments. These results suggest that the level of BUdR substitution has a minor role in determining the frequency of SCEs induced by BUdR. The relative roles of BUdR concentration and substitution in determining the frequency of SCEs can be judged by comparing the SCEs induced by 180 μM BUdR alone with those induced by FUDR plus 12 μM BUdR but without dT. Although the levels of BUdR substitution were the same in both conditions (~95%), the frequencies of SCEs were quite different (Table 1, parts 2 and 3). Clearly, the concentration of BUdR, not the level of BUdR substitution, was the major factor. Furthermore, as shown in Fig. 2, the ratio of exogenous BUdR molecules:cell number did not have a significant effect on the frequency of SCEs, in contrast to previous reports^{8,9}.

The results in Table 1, part 2, showed that increasing the level of BUdR substitution while maintaining the concentration of

**Fig. 1** Effect of BUdR concentration on the induction of SCEs.

For the determination of SCEs, tissue culture dishes (100 mm diameter) were inoculated with 5×10^5 CHO cells in 9 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (E medium). After several hours at 36.5 °C, 1 ml E medium containing BUdR and other additives at 10 times the desired final concentration was added to each dish. After 36 h, colcemid (0.06 $\mu\text{g ml}^{-1}$) was added, the cells were collected 2 h later, stained with 33258 Hoechst dye¹⁰, exposed to visible light and stained with Giemsa as described previously¹¹. Each point represents the average frequency of SCEs per cell for at least 20 cells. For the determination of mutant frequencies, dishes (100 mm diameter) were inoculated with 1×10^5 cells in 9 ml E medium, and the cells were exposed to BUdR and other additives for 36 h as described above. After the exposure to BUdR, the cultures were rinsed twice and renewed with 20 ml E medium containing 0.1 mM hypoxanthine and 16 μM dT (HT medium). After 5 days growth in HT medium, to allow for the expression of any mutations induced, the cells were collected and dishes (150 mm diameter) were inoculated with 2×10^5 cells in E medium containing 18 μM 6-thioguanine (TG). Dishes (60 mm diameter) were also inoculated with 250 cells in E medium alone. After 7 days, the cultures were fixed and stained, and colonies with more than 50 cells were counted in triplicate dishes. The results are expressed as the number of TG^r colonies per 10^5 cells, after correcting for the plating efficiency of the cells in the absence of TG. For the determination of BUdR incorporation into DNA, dishes (150 mm diameter) were inoculated with 10^6 cells in 45 ml E medium. After several hours, 5 ml E medium containing BUdR and other additives at 10 times the desired final concentration was added. Twelve hours later, 2 $\mu\text{Ci ml}^{-1}$ $\text{H}_3^{32}\text{PO}_4$ was added. After a further 36 h, the cells were collected, the DNA isolated and the base composition of the nuclear DNA determined as previously described¹⁷. Four determinations were made for each sample. The results are expressed as 'BUdR substitution', the percentage of dT residues in nuclear DNA replaced by BUdR. To avoid effects due to the photosensitivity of BUdR substituted DNA, the cells were protected at all times from wavelengths of light below 550 nm¹⁷. The cells were exposed either to BUdR alone (●) or to BUdR in the presence of 10 μM FUDR and dT (FBT medium) with the ratio of BUdR:dT fixed at 3:2 (○). The numbers by the data points indicate % BUdR substitution.

BUdR fixed had a small but apparently real effect on SCE induction. However, it can be seen that increasing the level of BUdR substitution in these conditions had no detectable effect on the induction of mutations. Thus, even though a reasonably good correlation between the induction of SCEs and mutations by BUdR was observed in most conditions (see Figs 1, 3), conditions exist in which the induction of SCEs and mutations by BUdR seem to be separable.

Because we had found that dC can suppress BUdR mutagenesis⁴, experiments were carried out to determine the effect of dC on BUdR-induced SCEs. These experiments were carried out in the presence of FUDR, to prevent the conversion of the exogenously supplied dC to dT nucleotides. As shown in

Fig. 3a, the addition of 200 μM dC largely suppressed the increase in SCEs due to increasing the BUdR concentration from 12 to 300 μM . Because of the presence of FUDR, this suppression occurred in the absence of changes in BUdR substitution. Comparable results were observed for the suppression of BUdR mutagenesis by dC (Fig. 3b), in agreement with previous results⁴.

Experiments were also carried out to test the ability of dC to suppress the induction of SCEs caused by increasing the level of BUdR substitution (from 35% to 88%) while maintaining a fixed concentration of BUdR (12 μM). In contrast to the results in Fig. 3a, dC had no effect on the induction of SCEs by BUdR in these conditions (Table 1, part 4). Thus, dC can suppress the induction of SCEs caused by increasing the concentration of BUdR, but apparently cannot suppress the induction of SCEs caused by increasing the level of BUdR substitution.

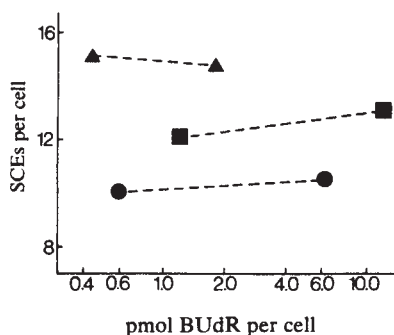


Fig. 2 Effect of BUdR:cell ratio on the induction of SCEs. The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in FBT medium with a 3:2 ratio of BUdR:dT. At a given BUdR concentration, the different BUdR:cell ratios were generated by varying the ratio of the volume of culture medium to the number of cells. At 30 μM BUdR (●) and 60 μM BUdR (■), cultures were tested at 5×10^5 cells per 10 ml (left point) or 1×10^5 cells per 20 ml (right point). At 90 μM BUdR (▲), cultures were tested at 1×10^6 cells per 5 ml (left point) or 5×10^5 cells per 10 ml (right point).

As the BUdR plus dye technique is being used to screen compounds for SCE induction, experiments were carried out to determine whether the conditions of BUdR treatment could affect the induction of SCEs by other agents. Cells were grown in FBT medium such that the frequency of SCEs was varied by changing either the BUdR concentration at a fixed BUdR substitution (60%) or the BUdR substitution at a fixed BUdR concentration (12 μM). The effect of mitomycin C (15 or 30 ng ml^{-1}) in inducing SCEs in the various conditions was determined, and in no case was there evidence for interactions between BUdR and mitomycin C. For example, in FBT medium with a 3:2 ratio of BUdR:dT, 30 ng ml^{-1} mitomycin C caused an increase of 96.9 and 95.3 SCEs per cell above the baseline for BUdR at 12 μM (9.3 per cell) and 180 μM (23.3 per cell), respectively. These results agree with those of a previous study in which BUdR concentration and substitution effects were not tested separately¹³.

The results presented above show that (1) the major factor in determining the frequency of SCEs induced by BUdR is the concentration of BUdR in the medium, (2) the amount of BUdR in DNA has only a small role in determining the frequency of SCEs, (3) the induction of SCEs by high concentrations of BUdR can be suppressed by dC without changing the amount of BUdR in DNA, and (4) the induction of SCEs by high levels of BUdR substitution is not suppressed by dC. Overall, there was a reasonably good correlation between SCEs and mutations,

although conditions to separate these two effects of BUdR were found.

The specific mechanism by which BUdR induces SCEs remains to be elucidated. However, the results presented above suggest that the primary effect of BUdR in inducing SCEs may

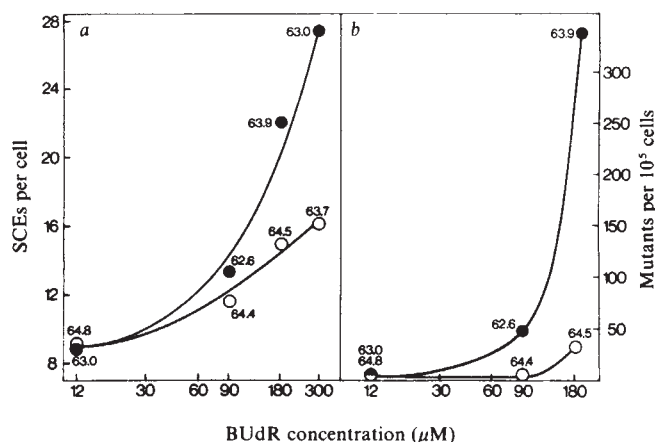


Fig. 3 Effect of deoxycytidine on the induction of SCEs by BUdR. The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in FBT medium with a 3:2 ratio of BUdR:dT. Half the cultures received 200 μM dC (○) at the same time that BUdR was added, the other half received no dC (●).

occur at a site outside DNA (although the ultimate target for SCEs is obviously DNA). One possibility raised by our results is that perturbation of dC metabolism has a key role in the induction of SCEs by high concentrations of BUdR, as previously suggested for BUdR mutagenesis^{2,3,14,15}. The induction of SCEs and mutations by high BUdR concentrations could involve a common site outside DNA, possibly the inhibition by BUdR triphosphate¹⁶ of the ribonucleotide reductase-catalysed reduction of CDP to dCDP. The fact that dC did not suppress the (small) increase in SCEs caused by increasing the level of BUdR substitution suggests that the independent effects of BUdR substitution and concentration on SCEs might involve different mechanisms. Of the two components of the effect of BUdR on SCEs, the minor component (BUdR substitution) may be directly related to the presence of BUdR in DNA, whereas the major component (BUdR concentration) seems to involve other sites.

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Influence of local vascularity on hormone receptors in mammary gland

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Procedures, such as teat removal (thelectomy) or teat duct ligation, which prevent removal of milk, lead to rapid involution of the lactating mammary gland; performed unilaterally they have been used previously to study the biochemistry of involution¹⁻³, enabling a comparison of normal and involuting glands in the same animal against the same systematic hormonal environment. Both the protein hormone prolactin and the steroid hormone oestrogen are of importance in the development and function of the mammary gland⁴. In the present experiments, female Sprague-Dawley rats were unilaterally thelectomised and the binding to the mammary gland of prolactin and oestrogen was examined through pregnancy, lactation and weaning. There was an effect of thelectomy during lactation only, when levels of both receptors increased in the intact lactating gland but failed to rise in the thelectomised, involuting gland. Capillary closure is known to occur in the mammary glands of rats after 36–48 h of milk accumulation⁵. The rate of delivery of hormones to the tissue will be drastically reduced and it is concluded that this, rather than systemic hormone levels, is of importance in controlling receptor levels.

Female Sprague-Dawley rats aged 34–45 days were unilaterally thelectomised under ether anaesthesia by ligation of milk ducts and excision of teats. Rats were allowed to recover for at least 21 days, and were then placed with a male for 4 days. The stage of pregnancy was confirmed at autopsy by inspection of pup development. The day of littering was day 1 of lactation. Litters were adjusted to 6 pups per dam and pups were weaned on day 21 of lactation. All animals were killed by stunning and immediate decapitation between 09.00 h and 11.00 h (lights on 08.00–20.00 h). Because of the small amounts of mammary tissue in virgin and some early pregnant females, mammary tissue from two or three animals was pooled for hormone binding measurements at these stages. Mammary glands were removed, placed on ice, blotted and weighed. The wet weight of mammary tissue reflected expected developmental changes, rising slightly between early and late pregnancy and increasing a further twofold in lactation in intact glands. Mammary weight differed between intact and thelectomised glands only during lactation. Thelectomised glands were about 60% of the weight of intact glands between 2 and 7 days post partum, falling to 40% in late lactation, equal to the weight in weaned rats, thereby demonstrating progressive involution.

To measure hormone receptors, mammary tissue was immediately cut into small pieces and homogenised in 3 volumes (w/v) of ice-cold buffer (0.01 M Tris-HCl, 0.0015 M EDTA, 0.012 M thioglycerol, pH 7.4, containing 10% glycerol, v/v), using 10-s bursts of a Silverson laboratory mixer-emulsifier at maximum speed with 30-s cooling periods until no solid material remained (three to six bursts). The total homogenate was centrifuged at 15,000g for 20 min (4 °C), the pellet and floating fat were discarded and the aqueous supernatant was centrifuged at 100,000g for 1 h. The supernatant (cytosol fraction) and the pellet (membrane fraction) were stored at –20 °C until assayed for oestradiol binding and prolactin binding respectively.

Table 1 Specific binding of ¹²⁵I-labelled ovine prolactin to intact and operated mammary glands of unilaterally thelectomised rats

Rats	¹²⁵ I-ovine prolactin bound (c.p.m. per 0.1 g wet weight mammary gland)		Significance
	Intact mammary gland	Thelectomised mammary gland	
Virgin (n = 4)	872 ± 193	643 ± 296	NS
Early pregnancy (1–11 days) (n = 6)	863 ± 538	1,585 ± 1,208	NS
Late pregnancy (12–22 days) (n = 9)	2,615 ± 2,054	1,768 ± 1,429	NS
Early lactation (2–7 days) (n = 6)	5,394 ± 1,446*	1,312 ± 1,109	P < 0.002
Late lactation (8–21 days) (n = 7)	6,531 ± 1,956	1,050 ± 778	P < 0.001
Weaned (5–12 days) (n = 6)	906 ± 808†	588 ± 338	NS

Prolactin binding was assayed in the membrane fraction (100,000g pellet) as previously described⁶. Ovine prolactin (NIH-P-S6) was iodinated to a specific activity of 22–48 µCi µg⁻¹ and 40,000 c.p.m. added to each assay tube. Values are means ± s.d.; n = no. of observations; NS, not significant, P > 0.05, Student's *t*-test.

* P < 0.001 versus intact mammary gland of virgin rats.

† P < 0.001 versus intact mammary gland of lactating rats.

The binding of prolactin (Table 1) and of oestradiol (Table 2) were measured in intact control mammary glands and in the contralateral thelectomised mammary glands of 9 virgin, 17 pregnant, 13 lactating and 6 weaned rats. Glands from virgin females showed low levels of prolactin and of oestradiol binding and there were no significant effects of thelectomy. Binding remained low during the early stages of pregnancy. After mid-pregnancy, prolactin binding tended to increase more in intact than in thelectomised glands but there were no significant differences between intact mammary glands of rats in early and late pregnancy or between intact and thelectomised mammary glands in late pregnancy. Oestradiol binding tended to be lower in late pregnant than in virgin females in both intact and thelectomised glands.

From the onset of lactation there were highly significant differences in prolactin binding between intact and thelectomised mammary glands. By 2–7 days post-partum, prolactin binding had increased in intact glands to two to three times the levels in late pregnancy, and five to six times the levels in the virgin gland, while in thelectomised glands, prolactin binding had returned to levels seen in virgin females. Prolactin binding in intact glands remained high throughout lactation and had returned to the levels in virgin females by 5 days after weaning, when they were not significantly different from the thelectomised glands.

Oestradiol binding increased in intact mammary glands in early lactation and continued to rise as lactation progressed, falling again to the levels seen in virgin females after weaning. In thelectomised glands there was no rise in oestradiol binding, which remained at the levels seen in late pregnancy.

The observations on binding of prolactin and oestradiol to the intact mammary glands confirm previous results in the rat showing marked elevation of both prolactin⁶⁻⁹ and oestradiol⁹⁻¹³ binding in lactation. The failure of prolactin and oestradiol binding to rise after removal of the young on the day of littering has been previously reported⁹, but in the present experiments it is striking that this occurred in the thelectomised glands of rats which were suckling pups on their intact glands and whose serum levels of prolactin would be markedly elevated for at least 15 days⁹. Oestrogen binding to rat mammary tissue is unaffected by ovariectomy¹¹, but increased by prolactin¹⁴. Prolactin binding is increased by prolactin itself and by oestrogen, acting at least in part by stimulating plasma prolactin levels (see ref. 6 for review).

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Table 2 Specific binding of ^3H -oestradiol to intact and operated mammary glands of unilaterally thelectomised rats

Rats	^3H -oestradiol bound (fmol per mg cytosol protein*)		Significance
	Intact mammary gland	Thelectomised mammary gland	
Virgin ($n = 4$)	10.8 ± 5.1	9.25 ± 4.75	NS
Early pregnancy (1–11 days) ($n = 6$)	6.7 ± 5.4	10.2 ± 10.3	NS
Late pregnancy (12–22 days) ($n = 9$)	4.9 ± 4.1	4.3 ± 5.7	NS
Early lactation (2–7 days) ($n = 6$)	$8.7 \pm 4.1^\dagger$	4.7 ± 3.8	NS
Late lactation (8–21 days) ($n = 7$)	20.9 ± 15.1	5.0 ± 3.7	$P < 0.02$
Weaned (5–12 days) ($n = 6$)	8.6 ± 4.1	3.2 ± 3.1	$P < 0.03$

Oestradiol binding was assayed in the cytosol fraction (100,000 g supernatant). Cytosol plus buffer (500 μl) was incubated overnight (5°C) with saturating amounts (2 pmol) of [6,7- ^3H]oestradiol-17 β (44 Ci mol $^{-1}$, Radiochemical Centre, Amersham) in the absence and presence of unlabelled oestradiol (2 nmol). Unbound steroid was removed by incubation at 0°C for 15 min with dextran-coated charcoal (1 ml 0.5% activated charcoal, Sigma, 0.05% dextran T80, Pharmacia). Charcoal was precipitated by centrifugation (1,500g for 10 min) and the supernatants decanted into 10 ml Instagel scintillation phosphor (Packard) and the radioactivity determined using a Packard TriCarb B2450 liquid scintillation counter, with automated external quench correction. The binding was specific for oestrogens being displaced by oestradiol and diethylstilboestrol, but not by testosterone, dexamethasone, cortisol or progesterone. Values are means $\pm$ s.d.; n = no. of observations; NS, not significant, $P > 0.05$.

* Protein measured by a modified Lowry method¹⁸.

$^\dagger P = 0.03$ versus mammary gland in late pregnancy.

When teats are blocked, ligated or removed, milk accumulates in the mammary gland. Early changes depend on whether the litter remains with the mother. If the litter is removed, then even in intact glands milk accumulates and mammary blood flow falls within 8 h as a result of reduced cardiac output and a reduced proportion of cardiac output being taken by the mammary gland¹⁵. If, as in the present experiments, suckling continues but milk removal is prevented, then even more milk collects in the tissue but the blood flow does not fall¹⁵. However, by 36–48 h capillary closure occurs in the mammary gland whether suckling occurs or not³, and there is marked reduction in metabolic activity^{1–3}. The collapse of the capillary bed can be seen by blanching of the tissue and was further demonstrated by Silver⁵ by failure of the gland to give a milk ejection response to oxytocin administered intravenously, whereas a response could still be obtained if oxytocin was applied locally to the exposed surface of the gland. Capillaries remained empty beyond 120 h in suckled glands⁵. As involution progresses, the proportion of cardiac output going to the mammary gland falls, apparently regulated locally by depressed mammary activity^{15,16}.

Our results indicate the importance of blood supply and rate of delivery of hormones to the tissue in the control of receptor levels. This could be of considerable importance in the regulation of receptor levels in breast cancer tissue, since angiogenesis, blood vessel development, is stimulated by neoplastic cells¹⁷.

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Novel expression-linked copies of the genes for variant surface antigens in trypanosomes

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Pathogenic African trypanosomes evade the immune system of their mammalian hosts by the sequential expression of alternative cell-surface glycoproteins (reviewed in refs 1, 2). Variant surface glycoproteins (VSGs) purified³ from cloned variants of *Trypanosoma brucei* have similar molecular weights (about 60,000), but differ in amino acid composition³, N-terminal amino acid sequence⁴ and C-terminal structure⁵. We have cloned DNA complementary to the messenger RNAs for four immunologically distinct VSGs⁶ and hybridised these complementary DNAs (cDNAs) with restriction digests of *T. brucei* nuclear DNA, fractionated by gel electrophoresis and transferred to nitrocellulose strips. Each cDNA recognises a unique set of fragments and this basic set is present unaltered in the nuclear DNAs from the four variants. In addition, each probe recognises an extra fragment only in nuclear DNA isolated from cells expressing the VSG corresponding to the cDNA probe. We infer that activation of a VSG gene involves the production of an expression-linked copy of that gene.

The cDNA clones used in our experiments were originally identified⁶ on the basis of their ability to hybridise only to poly(A)⁺ RNA from the homologous variant and this specificity is further documented in Fig. 1. Total poly(A)⁺ RNA from all four variants of *T. brucei*, strain 427, was size-fractionated by agarose gel electrophoresis (Fig. 1a), covalently linked to paper⁷ and then hybridised with one of the four cloned cDNAs. Figure 1b shows that cDNA of variant 117 only hybridises with homologous RNA; the same was found with the other three cDNAs (not shown). The mobility of the RNA species that hybridise is variant dependent (Fig. 1c), the calculated size varying from 2,250 nucleotides (variant 117) to 1,950 nucleotides (variant 221)⁶. This variation correlates with the apparent molecular weights of the corresponding pre-VSGs, deduced from their mobilities in SDS polyacrylamide gels. These decrease in the same order, 117 being 62,000, 221 being 52,000 (ref. 6).

The genes corresponding to these VSG-specific cDNAs were analysed by the Southern blotting technique⁸. Nuclear DNA from one *T. brucei* variant was digested with various restriction endonucleases, size-fractionated by agarose gel electrophoresis, blotted on to nitrocellulose strips and hybridised with each of the four cDNAs. Figure 2 shows results with three of these. Each

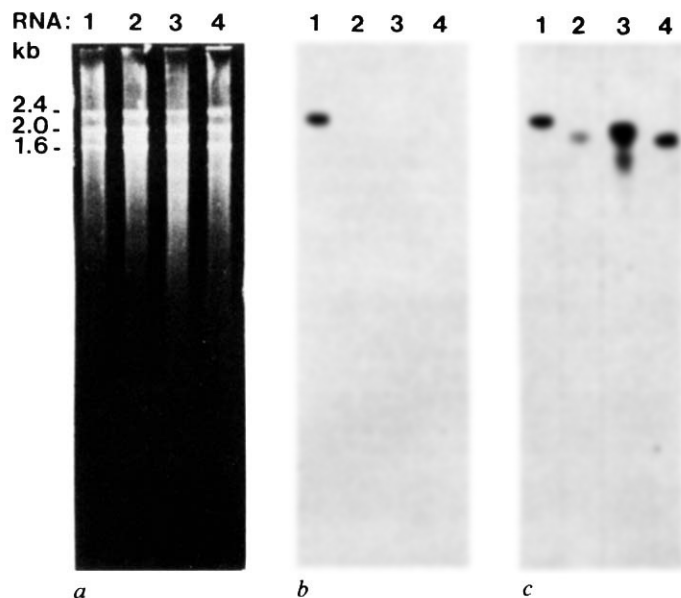


Fig. 1 Hybridisation of cloned cDNA with poly(A)⁺ RNA from the homologous variant and other variants. Glyoxal-treated poly(A)⁺ RNA (2 µg) from each of the four trypanosome variants was electrophoresed through a 1.75% agarose gel in Tris-borate buffer¹⁶. After transfer of the RNA to diazobenzoyloxymethyl paper⁷, the filters were hybridised with nick-translated recombinant plasmid DNA containing sequences complementary to VSG mRNA⁶. *a*, Photograph of the ethidium-stained gel after electrophoresis; lane 1, variant 117 RNA; lane 2, 118 RNA; lane 3, 121 RNA; lane 4, 221 RNA. The large rRNA contains an internal break and yields two bands. *b*, Autoradiogram of the RNA in *a* after transfer to diazobenzoyloxymethyl paper and hybridisation with a recombinant plasmid containing DNA complementary to VSG 117 mRNA (T-cV117-1). *c*, As *b*, but each RNA hybridised with the homologous cDNA probe; lane 1, T-cV117-1; lane 2, T-cV118-2; lane 3, T-cV121-3; lane 4, T-cV221-12. (Figure assembled from different replica gels.) The recombinant plasmids were made by inserting duplex DNA complementary to VSG mRNA into the *Pst*I site of plasmid pBR322 by the dG:dC tailing technique⁶. The sizes of the inserts are (in base pairs): T-cV117-1, 820; T-cV118-2, 1,500; T-cV121-3, 800; T-cV221-12, 1,200. See ref. 6 for isolation of poly(A)⁺ RNA, plasmid isolation, ³²P labelling of DNA by nick-translation, gel handling and other evidence that the cDNA inserts of these four plasmids have no sequence homology under standard hybridisation conditions.

hybridisation of each cDNA probe with homologous and heterologous nuclear DNA digests. Figure 3 shows results with the 118 cDNA probe. In each digest this probe sees one DNA fragment more in the homologous 118 nuclear DNA than in the heterologous 117 DNA. We attribute the fragments common to the 117 and 118 DNA digests to a basic copy of the 118 VSG gene, present in both variants. The results of double digestion experiments with two restriction enzymes fit a *Pst*I × *Eco*RI × *Bam*HI × *Hind*III map of this basic copy which accounts for all

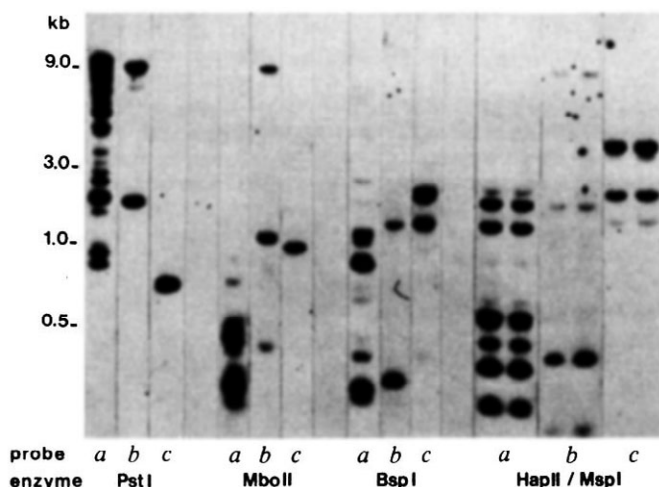


Fig. 2 Autoradiogram of variant-specific cDNA probes hybridised to restriction enzyme digests of nuclear DNA from *T. brucei* variant 118. Nuclear DNA was isolated by a modification of the kinetoplast DNA isolation procedure¹⁷, digested in standard conditions¹⁷ with the enzyme indicated and electrophoresed through a 0.6% agarose gel¹⁷. The gel was blotted on to nitrocellulose filter strips as described elsewhere⁸ and hybridised with nick-translated DNA from the recombinant plasmids indicated. See Fig. 1 legend for further details. Probes: *a*, T-cV117-1; *b*, T-cV118-2; *c*, T-cV121-3.

fragments seen in blots without invoking intervening sequences or the presence of more than one basic 118 VSG gene. In each digest shown in Fig. 3 the 118 cDNA probe hybridises to one extra band in the homologous 118 nuclear DNA, not present in the 117 nuclear DNA. An extra band was also found in the *Mbo*II, *Hap*II and *Msp*I digests (not shown). We attribute these extra bands to an additional expression-linked copy of the 118 VSG gene only present in 118 nuclear DNA.

Analogous results were obtained with the 117 and 121 cDNA probes. Figure 4 shows that the fragments hybridising with the 117 cDNA are identical except for an extra band in the *Msp*I digest of the homologous 117 nuclear DNA. Extra bands only in homologous DNA were also observed in the *Hap*II, *Eco*RI and *Pst*I digests (not shown), but not in *Bsp*I (Fig. 4) and *Mbo*II digests (not shown). The fact that an extra band does not appear in all digests of homologous DNA is not surprising. If the sequences around a gene change, this will only result in altered fragments in blots if the enzyme used cuts far enough from the gene segment recognised by the probe to include the altered sequence. The results with the 121 cDNA are more complex. In this case extra bands are observed in both the 121 and the 221 nuclear DNAs, but the extra bands are not identical in size. It is of interest that variant 221 has arisen during *in vitro* culture from variant 52, which is immunologically identical to variant 121 (ref. 10). The extra band in the 221 digest could, therefore, represent an inactivated form of the expression-linked copy of the 121 gene, but this is only one of several possibilities.

Analysis of several blots like those shown in Fig. 3 has yielded no reproducible differences in the relative intensities of the

cDNA hybridises to a different set of bands, confirming that each probe 'sees' different genes, as anticipated from Fig. 1. The 118 and 121 probes hybridise mainly to one or two bands in each digest, indicating that they primarily recognise one gene. At long exposures other faint bands become visible, suggesting that the probes cross-hybridise to other genes that show partial cross-homology with the 118 and 121 genes. The 117 cDNA probe hybridises to many different bands in all digests. We attribute this to extensive cross-hybridisation to a family of related VSG genes, because more stringent washing of the hybrids (in 0.1 × SSC at 60 °C) removes the label from all but a few of these bands (not shown). An even more complex pattern has been found with the 221 cDNA (not shown).

There are no differences between the *Hap*II and *Msp*I digests for any of the probes. Both enzymes recognise the sequence CCGG, but cleavage by *Hap*II is prevented by methylation of the C in the CpG doublet, whereas cleavage by *Msp*I is not⁹. Partial methylation of C in the CpG sequence, therefore, does not complicate the digests shown here.

To determine whether expression of a VSG gene results in sequence alterations in or around that gene, we compared the

US COLLEGE TEXTBOOK SUPPLEMENT

Bringing physics to life

Daniel Zwanziger

Physics (Formerly Life Science Physics). By Joseph W. Kane and Morton M. Sternheim. Pp.664. (Wiley: New York and Chichester, UK, 1978.) £11. *General with Bioscience Essays*. By Jerry B. Marion. Pp.539 plus index. (Wiley: New York and Chichester, UK, 1979.) \$18.95. *Physics for Applied Biologists*. By N.C. Hilyard and H.C. Biggin. Pp.223. (University Park Press: Baltimore, 1977.) \$13.75. *Intermediate Physics for Medicine and Biology*. By Russell K. Hobbie. Pp.557. (Wiley: New York and Chichester, UK, 1978.) \$23.95.

It would be hard to overestimate the degree of penetration of the concepts of physics into biology. On the one hand, the conceptual barrier between the living and the non-living world has continually eroded as our detailed knowledge of biological processes has increased. On the other hand, the introduction of new instruments and techniques resulting from advances in physics has in many cases opened up whole new areas of biology. Today's laboratory investigators use sophisticated instruments to study the function of an organ, cell or macromolecule which is ultimately understood by means of a simple physical concept such as diffusion, electrical conduction or tunnelling through a potential barrier. The two principal themes are entropy and energy which distinguish spontaneously occurring processes, namely those accompanied by an increase of entropy from active or pumped processes where entropy decreases at a cost of energy. The detailed knowledge of living things achieved by present day biologists is a triumph of the mechanistic world view in which, to be sure, quantum mechanics plays an important role. It is well recorded in Jacques Monod's broadly painted overview of biology *Le Hasard et la Nécessité* (Editions du Seuil, Paris, 1964).

It is fortunate that a number of texts have appeared to meet the need of the biologist to learn physics. Outstanding among these is *Physics with Illustrative Examples from Medicine and Biology* (Vol. 1: Mechanics by G.B. Benedek and

F.M.H. Villars; Vol. 2: Statistical Physics by F.M.H. Villars and G.B. Benedek; Vol. 3: Electricity and Magnetism by G.B. Benedek and F.M.H. Villars, Addison-Wesley: London and Reading, Massachusetts, 1973, 1974, 1979). These volumes are a milestone of pedagogy. They successfully integrate the history of the subject and the analysis of key experiments into its logical exposition. (This history reveals, incidentally, the corresponding debt of physicists to physiologists such as Volta, Mayer and Helmholtz, who in fact have made many of the important discoveries of physics.) These volumes provide a wealth of anatomical and physiological applications worked out in beautiful and patient detail. In the opinion of this reviewer they provide the best introduction available to statistical physics as pure physics, entirely apart from biological applications. Any text on the subject must be measured against the standard which they set. It seems, however, that the blinding light of truth cannot be revealed all at once to the uninitiate, and

that the content of these volumes is too rich for a first introduction to physics for those whose main interest is in the life sciences, and who generally take only two semesters of college physics. However, they make a wonderful text for an intensive three-semester introductory course in physics if the students are reasonably well motivated.

A number of texts have been published in recent years for a two-semester introductory course in physics with applications in the life sciences. Of those which this reviewer has examined, perhaps a dozen in all, the best by far, in his opinion, is *Physics (Formerly Life Science Physics)* by Kane and Sternheim. Most of these texts, written by physicists for the pre-medical student market, are basically standard physics texts with occasional applications of biology. The conspicuous virtue of the text by Kane and Sternheim, which distinguishes it from its competitors, is the breadth of coverage of the many subfields of physics and the remarkable completeness of the quantitative applications to the life

Sir John Kendrew and his model of myoglobin — the first protein to yield its structure to the physics of X-ray diffraction.

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sciences. The latter, taken together, could constitute the core of an introductory course in biomechanics and biophysics. The authors achieve this without sacrificing the derivations which are the meat of any course in physics, by clear and concise writing which allows them to present more on each page than the other texts. The wealth of material may be used selectively to advantage emphasising either the basic physics or the life science applications. Derivations which use calculus are segregated to the end of each chapter. There are abundant homework problems of graded levels of difficulty.

General Physics with Bioscience Essays by Jerry B. Marion is a text for a one-year non-calculus terminal course in physics. Though only slightly shorter than *Physics (Formerly Life Science Physics)* by Kane and Sternheim, it contains fewer derivations and fewer and less detailed life science applications, with no corresponding gain in clarity.

Physics for Applied Biologists by N.C. Hilyard and H.C. Biggin is a useful compendium of formulae and diagrams of instruments. However, to this reviewer's mind it is not really a physics text at all because derivations, as examples of physical reasoning, are almost entirely lacking. If physics-for-biologists texts become distinguished from pure physics texts by the absence of reasoning, they will

contribute to the division of the house of science instead of its unity. The phenomenon is before us in the statistics-for-social-scientists texts, generally written by social scientists instead of mathematicians, in which the subject is deprived of its logical structure and reduced to a verbose hodge-podge and unrelated formulae, because it is assumed that the student is incapable of understanding a logical derivation. The vicious circle is fatally closed when the student does indeed conclude that he is incapable of understanding statistics.

Intermediate Physics for Medicine and Biology by Russell K. Hobbie brings together material not readily available elsewhere. It is the only text on the subject, which this reviewer has seen, in which a knowledge of calculus and a previous acquaintance with physics are assumed. The author was motivated by the sophisticated level of physics applications in medical school courses and the poor preparation in physics prevalent among medical students. He has an ambitious aim, namely a logically complete though concise presentation of physics, without reliance on "it can be shown", though the main body of the text is devoted to applications in the life sciences. The result is successful. For example, the brief introduction to statistical physics is applied effectively in subsequent chapters to the phenomenon of

osmotic pressure and transport through membranes. Similarly, sufficient electricity and circuit theory are presented for a cogent exposition of the Hodgkin-Huxley model of nerve conduction and the electrocardiogram. The quantum effect in dark-adapted vision, discovered by Hecht, Schlaer and Pirenne, is clearly explained. It would be helpful if, in a new edition, more anatomically correct drawings accompanied the physicist's schematic or block diagrams. This text is both competitive with and complementary to the volumes by Benedek and Villars which I praised above. There is considerable overlap of subject matter, but Hobbie assumes greater background on the part of the student and thus can be more concise. His book would make an excellent text for an upper division undergraduate course. Such a course could profitably be introduced in many universities. For physics students it would be a valuable introduction to applications in the life sciences which may offer them interesting career opportunities. It would be invaluable for graduate students preparing for research in the life sciences, where the concepts of physics have assumed overwhelming importance. □

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Physics starts here; or here?

David J. Miller

Concise Dictionary of Physics. By J. Thewlis. Pp.366. (Pergamon: Oxford and New York, 1979.) £20.00. *Introductory Physics.* By M.L. Warren. Pp.683. (Freeman: San Francisco and Reading, UK, 1979.) £20.50. *Physics.* By Jay Orear. Pp.752. (Collier-Macmillan: London, 1979.) £13.50.

SHOULD physics be taught as a body of facts or as a system of ideas? These three books at introductory college level reflect the most divergent of views.

The *Concise Dictionary of Physics* claims in its preface to be intended not only for students but also for teachers, engineers, journalists and for the interested layman. It is surely a useful compilation; by no means comprehensive but amazingly good for 370 pages, and admirably concise. In sampling an arbitrary list of topics which I had decided in advance it ought to contain only one was missing — Clebsch-Gordan coefficient. There are few inaccuracies, though baryons are no longer those "fermions with mass greater than or equal

to that of the nucleon". The chief criticism to be made is not of the dictionary's accuracy but of its relation to the rest of the world of physics. Concepts here are "formulated, sprawling on a pin", lifeless and unconnected with the world in which they live. When so much trouble has been taken to write the definitions it seems perverse not to offer references to textbooks, or bibliography. I would like to know what a "quaternion" is, for instance. Where do I go next after reading that it is "an operator, in a system of vector analysis invented by Hamilton, which changes one vector into another by rotation accompanied by a change of magnitude"?

Jay Orear's *Physics*, an introductory textbook for science and engineering students, goes to the other extreme. He begins by saying that the goal of physics is "to seek out and understand the basic laws of nature upon which all physical phenomena depend." The book reflects this statement, with a methodical progression from mechanics to special relativity, followed by heat and thermodynamics, then electricity and magnetism, waves and optics, quantum physics, atoms, molecules, solids, nuclei, astrophysics and particles. It is assumed that students will learn calculus in parallel with this course, so he is able to present the "basic laws of nature" in their

mathematical form. This is not a book for biologists or medical students, but physics students will want a more complete account of each of the subjects introduced here. In 752 sparsely laid out pages there is no room for detailed experimental justification of many of the assertions made and there are surprisingly few illustrations. Its use will probably be in those universities where an introductory physical-sciences class is given high powered courses in mathematics, physics and chemistry before separating into specialised groups. Compared with a rival such as the three-volume *Fundamental University Physics* by Alonso and Finn (Addison-Wesley: Reading, Massachusetts and London, 1967), this book has the advantages of being up to date and short enough for the logical structure of the whole subject to be clear. There are twenty or thirty problems at the end of each of the thirty chapters, with answers to the odd-numbered problems at the back. A bibliography of more specialised texts would have been useful.

Introductory Physics by Mashuri L. Warren is aimed at biologists and medical students. My only objection to it is that its organisation is sometimes more like that of Thewlis' dictionary than can really be justified in a textbook. Where Orear never loses sight of physics as a unified system of ideas, Warren treats it as a body of often

unconnected facts. A strange example is the treatment of the equivalence of mass and energy, attributed to Einstein and discussed in some detail, but in no way related to the subsequent chapter on the special theory of relativity. But, having grumbled a little, let me say what a useful book it is. It crams an enormous amount into 683 double-columned pages, with line drawings or photographs of equipment on every page. Because the student is assumed weak in mathematics, most results quoted

are backed up by generous experimental — even anecdotal — detail, as well as some basic mathematical exposition. Physics for its own sake gets little space — particle physics hardly figures at all — but there is a chapter on electric shocks and electrical safety which taught me a great deal that I should have known before; and the chapters on optics and hydrodynamics are sensible and detailed. There are twenty or thirty simple problems at the end of each chapter, with answers to the odd numbers

at the back. The bibliography for each chapter is eclectic — not many specialised textbooks but a range of historical material, memoirs of scientists and *Scientific American* articles. There are appendices on basic mathematics, physical constants and units, “well known functions” and problem-solving with calculators. □

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Remote sensing of the environment

R.N. Colwell

Introduction to Remote Sensing of the Environment. Edited by Benjamin F. Richason Jr. Pp.496. (Kendal/Hunt: Dubuque, Iowa, 1978.) \$27.00. *Laboratory Manual for Introduction to Remote Sensing of the Environment.* Edited by Benjamin F. Richason Jr. Pp.237. (Kendal/Hunt: Dubuque, Iowa, 1978.) \$8.95.

THESE two books are companion volumes prepared by 24 contributors under sponsorship of the National Council for Geographic Education and published in the Council's Pacesetter Series.

The first chapter of the textbook provides a stage-setting overview. The second chapter describes the nature of electromagnetic energy and of basic matter/energy relationships. The next eight chapters describe various remote sensing devices and aerospace image acquisition systems; included are illustrations of the interpretation of black-and-white, colour, and colour infrared photographs, and of imagery acquired by thermal infrared scanners, multispectral scanners and microwave sensors. The next nine chapters deal with specific remote sensing applications including the analysis of landforms, rural cultural landscapes, agriculture, forestry, urban and industrial areas and weather phenomena; cartographic and regional planning applications are also included. The concluding chapter constitutes both a summary and a look to the future. The book's four appendices list sources from which remote sensing imagery and data tapes can be obtained, provide reference materials for beginning students, and present colour reproductions of various types of imagery. They are followed by biographical information about the contributing authors and a brief subject index. A much needed glossary is not included. The laboratory manual closely parallels the textbook, with appropriate emphasis on applications.

The scope of these two volumes is

excellent and the concept of multiple authorship is commendable. The quality with which the image examples are reproduced ranges from good to excellent and most of the writing is highly articulate. Closer study of the textbook suggests, however, that its chapters comprise more a compendium of separate treatises than a well integrated introduction to remote sensing. One manifestation of this is a lack of standardisation of terminology. For example, is “remote sensing” (the most fundamental of terms) limited to the mere “imaging of features”, as defined in the Preface? Does it include the obtaining of digital records, as implied later in the text? In addition to data acquisition, is data analysis encompassed by this term? If so, does the analysis encompass work done not only by humans but also by machines? With respect to balance: (1) symptomatically, the chapter on agriculture planning is given roughly four times this emphasis in both respects; (2) of the 24 contributors, no less than 20 appear to be geographers; (3) more than half of the 24 colour plates pertain to features in the Great Lakes area; and (4) while the captions for a few of these colour plates are quite complete and are well cross-referenced to the two books, the captions for others indicate only the image type and/or geographic location.

Superficially, the laboratory manual is

very impressive, well illustrated and well matched to the supporting textbook. On a closer look, several of the exercises appear decidedly superficial. Also, the limited use of aerial photo stereograms gives the erroneous impression that three-dimensional perception and interpretation are of little importance.

There are notable strengths to offset these deficiencies. For example, Chapter 2 provides a masterful disclosure of some very important but highly complex material at a level that can be readily comprehended by most beginning students. Similarly, Chapter 16, dealing with regional planning, is clearly expressed, reasonably comprehensive, well balanced and well illustrated.

In summary, these two books represent a commendable effort to satisfy a long-standing need, that of providing the beginning student with a reasonably comprehensive remote sensing textbook and laboratory manual that might be digested in a single four- or five-unit course at the college or university level. A more carefully edited and better balanced second edition would far better satisfy this need. □

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Practical organic chemistry

J.E. Girard

Practical Organic Chemistry: A Basic Course. By V.V. Nekrasov. Pp.410. (MIR Publishers: Moscow, 1978.) \$7.80.

THIS fifth edition of a popular Russian text has been translated into English by Alexandar Rosinkin. What is not evident from the title of this book is that it is a laboratory text for students at vocational or technical school or for non-chemistry university students. As the author states in the introduction, this text is intended to be “only a practical manual, and it cannot rival the textbook of organic chemistry.

The theory is given in a limited scope”.

For the most part, the chapters are arranged according to functional groups. Within chapters are experiments dealing with qualitative organic analysis and simple synthesis. The latter chapters deal with polymer reactions, precipitation and denaturing proteins, hydrolysis of fats, preparation of mustard oils and reactions of carbohydrates.

Although this is a very thorough treatment, it is somewhat out of date. The translator has unfortunately chosen to use words or phrases that are not commonly used. This may cause beginning students undue confusion. □

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Physical chemistry texts

David S. Kliger

A Textbook of Physical Chemistry. Second edition. By Arthur W. Adamson. Pp.953. (Academic: New York, 1979.) \$22.95. *Physical Chemistry.* Fifth edition. By Robert A. Alberty and Farrington Daniels. Pp.682. (Wiley: New York, 1979.) \$19.50. *Physical Chemistry: A Step-by-Step Approach.* By Marwin K. Kemp. Pp.1034. (Marcel Dekker: New York, 1979.) \$23.75.

In *A Textbook of Physical Chemistry* Adamson has put some new twists into the traditional format of physical chemistry texts. The ordering of topics, for instance, is significantly altered; a chapter on additive physical properties of matter appears near the beginning of the book. This chapter covers absorption of light, molar refraction, molar polarisation, and dipole moments. These topics would normally be found much later, after discussions of molecular structure. Another novelty is the placement of statistical mechanics in this text. Rather than devote a separate chapter to this topic Adamson includes discussions of statistical mechanics in chapters on thermodynamics. This makes the link between statistical mechanics and thermodynamics more apparent than in many texts.

Other non-standard features of this text include 'Commentary and Notes' and 'Special Topics' sections at the end of each chapter. These contain expansions of topics covered in the chapter, discussions of historical points of interest, and brief discussions of related topics. Unfortunately, some of these sections discuss material much more advanced than material covered in the related chapter. Also, many of the 'Special Topics' are topics normally discussed in physical chemistry texts. Here they are merely added at the end of chapters rather than integrated into the main text.

Overall, this book is a rigorous, rather sophisticated text that would make a fine reference source. It has a fine index and many good figures which help clarify the text. As a book to introduce students to the subject of physical chemistry, however, it seems too sophisticated. Many discussions are too advanced and the ordering of topics sometimes makes the material difficult to understand. There are many problems for students to solve, separated into sections of straightforward exercises, demanding problems, and special topics problems. These are fine learning tools for students. For all but the most sophisticated student, however, the main text will probably be difficult to follow.

Physical Chemistry, by R.A. Alberty and F. Daniels, is a rather traditional

textbook for a year-long physical chemistry course. It presents little history behind the subject areas or motivation for studying them. Instead, the emphasis seems to be on presenting formulae to be used in a physical chemistry course. This may be reasonable for use in a course based on a lecture format where the instructor provides background and motivation. This approach leads, however, to a rather dry text. Consistent with this format of a text to supplement lectures, the book presents many problems and answers to about half of them. This is probably the strongest feature of the book from the instructor viewpoint.

Treatments of various topics in the book vary greatly. Many topics are presented too tersely, making it seem that learning facts and formulae is more important than understanding underlying principles. This seems particularly true of the coverage of quantum theory and, to a lesser extent, of thermodynamics. Later sections covering Statistical Mechanics and Kinetics, however, are quite well done.

Some topics covered in this book, such as equilibrium for biochemical reactions, macromolecules, and surface thermodynamics, are not found in most textbooks of physical chemistry. They add a very nice supplement to the traditional topics found in most texts. On the other hand, these chapters again tend to emphasise facts rather than present a general overview of these special topics.

In all, this book would be useful in courses where lectures provide the primary source of fundamental material. It does not, however, stand on its own as a primary learning tool for physical chemistry.

Physical Chemistry: A Step-by-Step Approach, by M.K. Kemp, differs from

other physical chemistry texts in that it is designed for use in a self paced (sometimes called 'Keller Plan' or personalised system of instruction) course. In such a course there are often no lectures and the book becomes the primary source of information for students. Thus, the book must be very clear. This book is just that.

Each topic in the book is introduced with a statement of the importance of understanding the topic as well as a list of learning objectives the student must keep in mind. After discussion of each topic there are problems, with detailed solutions given in an appendix, and a self test. A detailed table of contents and a list of symbols and definitions also precede each topic to aid the student.

This book has very few weak points. There are not as many problems as one might wish and the introduction to quantum theory is weak. However, later sections on atomic structure and spectroscopy are excellent. Most topics are introduced at a very elementary level and progress through to a rather high level of sophistication. Treatments of real gases, colloid and surface chemistry, and polymers are particularly thorough.

In all, this is an outstanding text for physical chemistry courses using a self paced format or a standard lecture format. *Physical Chemistry*, by P.W. Atkins (Freeman: San Francisco, 1978), is the only other physical chemistry text this reviewer has seen which matches this book in clarity of presentation. □

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Physical chemistry for the life sciences

R.M. Harris

Physical Chemistry with Applications to the Life Sciences. By David Eisenberg and Donald Crothers. Pp.868. (Benjamin Cummings: Menlo Park, California, 1979.) \$21.95.

THIS book surpasses all other life science-orientated physical chemistry texts I have examined in both the extent and depth of its coverage of traditional physical chemistry topics. Thermodynamics, solutions, electrochemistry, statistical mechanics and X-ray structure analysis are all presented at about the same conceptual and mathematical level as found in standard 'for chemists' texts, such as the most recent edition of *Physical Chemistry* by Moore (Prentice-Hall: Englewood

Cliffs, New Jersey, 1972). This is not to say it could replace a text like Moore's in a traditional two-semester 'for chemists' course. Its treatment of atomic structure and chemical bonding is neither extensive nor rigorous enough, and it has little to say about gas properties, kinetic molecular theory or reactions in the gas phase.

This text also has much to offer in the way of biological applications. Along with the usual topics (enzyme kinetics, transport properties, membrane equilibria, bioenergetics) it provides a more extensive treatment of macromolecular biophysics, including DNA characterisation, than any of the competing texts I know of, and it contains a lengthy qualitative section on the application of symmetry to the description of biological systems. Another attractive feature is that many of the mathematical results derived in the text are accompanied by a verbal description of their physical significance, and in most instances the explanations successfully illuminate the equations.

I was disappointed to find that several important biochemical topics normally included in a text of this kind were omitted by Eisenberg and Crothers. Aside from a paragraph on transition-state analogues, there is no discussion of enzyme inhibition, and there is nothing in the way of photochemistry or photobiology apart from a passing reference to photosynthesis. I also feel that the text needs more illustrations, and many that are present are too small or too sketchy. In

reading the sections on membrane phenomena, for example, I missed not seeing a picture of the modern model of a biological membrane, and the illustration accompanying the discussion of the hydrophobic effect did not help me at all in envisioning 'icebergs'. My most serious concern is that some of the major topics are developed in an unnecessarily formal manner. The reader has to labour through an entire chapter on principles of spectroscopy, and several sections of the

ensuing chapter, before encountering illustrative chemical examples.

Overall, the book's good points outweigh its shortcomings. You will want to give it serious consideration if you are seeking a text that offers life science students a solid foundation in physical chemistry. □

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Biology from a human angle

M. Edidin

Inquiry into Life. Second edition. By Sylvia S. Mader. Pp.771 plus appendix, glossary, index. (Wm. Brown: Dubuque, Iowa, 1979.) \$15.95. *Biology. Human Perspectives*. By Charles Kingsley Levy. Pp.562. (Goodyear: Santa Monica, California, 1979.) \$17.95. *Man, Nature and Society. An Introduction to Biology*. Second edition. By E. Peter Volpe. Pp.662. (Wm. Brown: Dubuque, Iowa, 1979.) \$15.95. *Biology. A Human Approach*. Second edition. By I.W. and V.G. Sherman. Pp.636. (Oxford University Press: 1979.) \$16.95.

ALL four of these books aim to introduce biology to students beginning a college education. It must be assumed that such students have no background at all and instruction must begin with the most basic and general aspects of biology. The field is notable, even to one who works in it, as short on theories and general organism principles and long on variety. The broad problems of life — maintenance, regulation and reproduction — are approached in a bewildering diversity of forms and treated in many different ways by living organisms. How does an instructor construct an introductory course from such diverse material? The books under discussion do so by concentrating on the biology of a single species, humans, discussing important generalities in terms of human examples and then continuing with discussion of specific problems or processes peculiar to the species and its relatives. Around 98% of all organisms are excluded by this approach.

The reasons for concentrating on the biology of a single species are variously stated by the authors. Mader's book, *Inquiry into Life*, "emphasizes the application of this knowledge to modern concerns . . . biology is truly relevant." *Biology. Human Perspectives* by Levy aims to avoid fragmentation and to con-

centrate on the biology of the most intensively studied organism. He, too, feels that this biology will have the greatest "appeal, relevance and interest . . ." for students, busy enquiring humans that they are. Volpe in *Man, Nature and Society. An Introduction to Biology*, wishes to educate liberally, to present biology so that intelligent use can be made of it by humans who, though not extensively trained in science, can recognise the importance of biology in many social and ethical problems in society and can approach such problems thoughtfully and rationally. The Shermans in *Biology. A Human Approach* similarly hope to present information that will enable students "to understand themselves and the world in which they live." They, too, consider personal relevance and "areas of immediate human concern" important to their presentation. All the books, then, wish to show us biology epitomised in ourselves and as affecting us in the world today.

The four books, though attempting more or less the same feat, do not approach the subject with the same intellectual views, principles of organisation or physical orientation. Also, they do not seem intended for the same students. The intellectual level of the texts varies greatly, as does the emphasis on the material presented.

Before commenting specifically, I ought to note my own prejudices. First, I am not sure that the human organism is so interesting or so dominant that it is worth an entire general biology book. Many humans think otherwise; three of the texts considered here are in second editions. Second, to my mind, the only truly integrating approach to general biology is an evolutionary and genetic approach. Without this, texts fragment into sections not easily connected to one another. The first and second prejudices reinforce each other. Third, I have been teaching a general biology course, using several different texts, over the past eight years. My reactions to new texts are dulled, rather than heightened by the experience.

Of the four books, by far the most interesting, easy to read and best organised was Volpe's. Rather than begin with a discussion of 'life' and its origins, or with a long section on chemistry, Volpe begins

with a discussion of human reproduction, nicely balanced between endocrinology and anatomy. This is followed by an outstanding chapter on the control of fertility in humans which in turn is followed by a clear descriptive discussion of vertebrate development and birth. A clever transition is then made to consideration of the biochemistry needed for modern biology by considering the problem of trans-placental transfer of nutrients. We then return to cell differentiation and development. The section is concluded with seven clearly written chapters on physiology and endocrinology of adults. Two sections follow on genetics, evolution and population genetics. These constitute nearly half the material in the book and take the student from the chemical basis of inheritance via biochemical genetics and simple population genetic studies of selection and evolution, to questions of polygenic inheritance, speciation and adaptive radiation. The concluding section covers other aspects of populations and biological communities. Appendices on simple chemistry and on probability follow the body of the text. Throughout the writing is clear; transitions between subjects are deftly made, and the material is accurately presented. The standard of illustration is high; both colour photographs and coloured full page drawings are used in addition to smaller black and white photographs and drawings. I regret only that this text, like most others in the field, does not supply scales to each picture. Biology deals with structures over at least a millionfold size range. Certainly, beginners ought to be helped to some sense of scale. Volpe's book is directed at intelligent beginners. It is first rate overall and should stimulate both students and teachers using it.

The three other texts considered here are rather more like each other than not. Sherman and Sherman and Levy present material in a traditional manner, emphasising physiology and structures far more than genetics and evolution, though the latter subjects do make an appearance. The Shermans' book is clearly written, though the use of boldface to introduce important words and phases makes the text rather shout at the reader. I concede that students in this country often apply liberal amounts of yellow highlighting to achieve

the same result, but I would rather shout myself than be shouted at. The quality of drawings and photos is not as high in this book as in Volpe's, but the drawings especially are clear. The text begins with a discussion of the origin of life and the methods of science, considers cell architecture, biologically important molecules and cell energetics. The first section closes with a discussion of the chemical basis of heredity and of cell division. A long second section is devoted to development and physiology and this is followed by a shorter section on mendelian and population genetics. Overall, the book is workmanlike, but I did not find it especially exciting. Its design also made it difficult reading. The book is printed largely in shades of blue ink, including the text itself. This is hard on the eye.

Charles Levy's book, the only one of the four making its first appearance, is

similarly organised, but the bulk of the book considers physiology and regulation. Genetics and development are given only two chapters at the end of the text, about 60 pages out of about 550. I do not think that this balance is correct, but those who wish to emphasise human physiology may find the text useful. However, it should be noted that there are a number of errors of fact in both text and drawings. The creation of a text is a difficult job and I think that allowance for errors should be made. Bright students will catch many of them (for example the jump from calories to kilocalories that occurs from pages 57 to 58). Physically, the book is somewhat smaller than the two others already discussed and this cramps the illustrations, which are often deployed in the margins of the text. The audience for this book as for the previous text is the group of average college students. They can learn from both

books through diligent application, but I am not sure how interested they will be in either of these texts.

The last book to consider, by Mader, is directed at a less sophisticated audience than the other three. It is written in a simpler style and readers are talked at, rather than being shown the development of an idea. There is, from my point of view, an excessive catering to students' needs for information on, and concern with, drug action, and with venereal diseases. A large quantity of biological information is contained in this book. The author is trying to deliver it to students who may be preoccupied with matters other than classwork. I am not certain that the approach emphasising 'relevance' to society is the one to use. □

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Primate cytogenetics

Dorothy A. Miller

The Phylogeny of Human Chromosomes. By Héctor N. Seuánez. Pp.189. (Springer: Berlin, Heidelberg and New York, 1979.) Paperback DM38; \$20.90.

CURIOSITY about the origin of man has led to the application to evolutionary problems of a variety of new techniques. *The Phylogeny of Human Chromosomes* by Héctor N. Seuánez deals with information derived from the use of the chromosome banding techniques that were developed during the past ten years.

The initial chapters present a brief overview of human evolution. This is useful as background, but the author fails to point out that chromosome studies can be carried out only on living organisms. Although we might like to know the karyotype of *Homo erectus*, comparisons must be made among species that exist at the present time. This volume, then, deals primarily with comparison of the karyotype of human with those of the great apes — chimpanzee, gorilla and orangutan. One of the results of comparative chromosome studies has been to place the gibbons and siamangs in a family separate from the great apes, with which they were classified at one time.

Brief descriptions of the major chromosome banding techniques are presented. G- and R-banding are used to identify every chromosome in the complement. (Q-banding is used for the same purpose, not just for identifying brilliant variant regions, as the author implies.) G-banded karyotypes of human, gorilla, chimpanzee (two

species) and orangutan are illustrated. Direct comparison of the various species would have been facilitated by presenting more composite karyotypes in which the presumptive homologues are placed side by side. It is unfortunate that the figures in chapter 7, where some of the chromosomes are placed together for comparison, are not arranged as effectively as are those in chapter 6 in which chromosomes of different orangutans are compared.

The most exciting finding in primate cytogenetics has been that every human chromosome has a recognisable counterpart in each of the four species of great apes. (This is in marked contrast to the results obtained in the rat and mouse, which are also evolutionarily related species but in which only a few chromosomes have similar banding patterns.) Some reorganisation of the genetic material has taken place during the evolution of the higher primates and man. There has been one fusion that results in the human having 46 chromosomes compared to 48 in the great apes. The most frequently observed differences between the chromosomes of the four species are the result of pericentric inversions, which involve a break on each side of the centromere of a chromosome, with rejoining of the inverted segment within the same chromosome. In a practical sense this has facilitated the comparative studies because single altered chromosomes are much easier to recognise than multiple reciprocal translocations. There is no evidence that the pericentric inversions which have become fixed in primate evolution have affected the activity of the genes on these chromosomes.

The demonstration that there are chromosomes with similar banding patterns in humans and great apes is of

much more trivial interest because these chromosomes often carry the same genes. Somatic cell hybrid methods have been used to show that one or more genes on almost every human chromosome has a counterpart on the purported ape homologue. In only one or two cases was a particular gene not carried by the chromosomes that had similar banding patterns in the various species. Similar comparisons with rhesus and African green monkey chromosomes show that genes carried by human chromosome number 1 have been associated with a chromosome segment having the same banding pattern for perhaps 35 million years.

The 18 and 28S ribosomal RNA genes are in a rather special class because they are present in hundreds of copies per cell rather than in 2 or at most a few copies per cell, as is true of most genes. Extensive redistribution of the rRNA genes has taken place during evolution. Most primates have these genes concentrated in a single site, but the orangutan has rRNA genes on 9 pairs of acrocentric chromosomes, the chimpanzee and human on 5 pairs (not all the same), and the gorilla on 2 (or 3) pairs.

Detailed comparisons are presented of the results obtained using methods that provide information about heteromorphic chromosome regions, many of which contain highly repetitive DNA sequences. Quinacrine-brilliant regions, for example, are found only in chimpanzee, gorilla, and human, and a quinacrine-brilliant Y is present only in the latter two species. Human satellite DNAs I, III and IV generally anneal to corresponding sequences on fixed metaphase chromosomes of each of the great apes, although to only a small extent in the orangutan. Human satellite II, on the other hand, anneals to gorilla and orangutan chromosomes but

not to those of the chimpanzee. If human satellite II-like sequences are present in the chimpanzee, they must be there in very small amounts, a finding that is supported by the absence of much 5-methylcytosine, a rare base which is abundant in human satellite II.

Dr Seuánez is at his best when describing his own studies with the orangutan, in which he has shown that animals from Borneo and Sumatra differ by a pericentric inversion (perhaps marking incipient species), but that each population is still heterozygous for a different complex

chromosomal rearrangement. In this volume he has included a wealth of information about a broad range of techniques. Students and those interested in primate cytogenetics will find the volume useful because it includes a large number of references, although a single reference list would have been easier to use than will be the separate list presented after each short chapter. General interest readers will probably be disappointed by the book because the broader aspects of phylogeny have been submerged in the sea of minute details. A final chapter drawing together

information derived from the various methods would have been a welcome addition. Most of the illustrations are well designed, but the chromosomes in some of the composite karyotypes are so small that they present little information. Unfortunately there are a large number of typographical errors which distract attention from the message of the text. □

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Basic molecular biology

G.C. Walker

Basic Molecular Biology. By Fred W. Price. Pp.497. (Wiley: New York, 1979.) \$20.50.

BASIC MOLECULAR BIOLOGY by F.W. Price is intended to be a suitable text for freshmen with a background in general biology and chemistry. The book opens with an extremely elementary introduction to basic chemical principles and then is divided into four main sections. The first section, which comprises almost half of the book, discusses primary through to quaternary structures of proteins, and the relationship of structure to function. One chapter provides a particularly clear general introduction to enzymes and others discuss topics such as fibrous protein assemblies and antibodies. The second section covers the structure of biomembranes and biological energy transducers. The third section covers the structures and roles of nucleic acids in the storage and expression of genetic information. A somewhat speculative chapter on chromatin structure is included. The final section is quite broad and considers the organisation of prokaryotic and eukaryotic cells.

In his preface, Price states that his approach is 'structural'. Indeed, almost every topic is introduced by a consideration of the relevant chemical and structural parameters of the molecule or assembly in question. Since the author has also intentionally minimised the attention paid to molecular genetics and protein synthesis, the book is much closer in many respects to a biochemistry text than to books such as *Molecular Biology of the Gene* (Watson; W.A. Benjamin: Menlo Park, California, 1976) or *Molecular Genetics* (Stent and Calendar; Freeman: San Francisco, 1978). In particular, while genetics is treated as a subject in one chapter, very few genetic experiments or

approaches are discussed elsewhere. Knowledge of basic organic chemistry is required throughout.

The book is clearly written and profusely illustrated. Occasional touches of humour help personalise the text. However, greater care in the relative emphasis given to specific examples would have improved the book. For example, several pages are devoted to the physical parameters of invertebrate oxygen-transporting proteins yet the discussion of ATCase in the section on allosteric enzymes is very short and has no illustrations. Other examples discussed, such as that of resilin, seem to contribute little to an introductory text.

Price also comments that, in writing an introductory text, he has resisted the temptation to delve deeply into certain exciting aspects of molecular biology. Nevertheless, I was disappointed that a number of the models discussed in detail seemed quite dated and were presented in an uncritical fashion, and also that such a recent book did not even allude to current topics such as recombinant DNA, DNA sequencing, the Ames test, or hybridomas. □

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Views of enzymes

Dennis Piszkiwicz

Steady-State Enzyme Kinetics. By Stanley Ainsworth. Pp.255. (University Park Press: Baltimore; Macmillan: London, 1977.) \$17.50. *Enzymatic Reaction Mechanisms.* By C. Walsh. Pp.978. (Freeman: San Francisco and Reading, 1979.) \$29.50. *Principles of Enzymatic Analysis.* Edited by H.U. Bergmeyer and K. Gawehn. Pp.260. (Verlag Chemie: Weinheim, 1978.) \$25.90.

In the past two decades our understanding of the nature and properties of enzymes has grown at a prodigious rate. The availability of texts describing these advances is now catching up with this growth. In recent years we have been treated to a large and somewhat redundant supply of texts on enzyme kinetics and mechanisms, albeit of varying scopes, lengths and quality. Three recent and different approaches to enzymology are examined here.

Kinetics observes catalysis in the time scale in which it occurs. It is the most important and popular tool available for the elucidation of enzymatic mechanisms.

Steady-State Enzyme Kinetics by Stanley Ainsworth presents an up to date survey of the mathematical gymnastics used to analyse enzyme kinetic data. It emphasises the methods of deriving models that describe the orders and methods of interaction of substrates and effectors to the catalytic molecules. The major fault of this text is that it really does not address the purpose of enzyme kinetics — the definition of the mechanism of catalysis — in as much detail as possible. It barely touches on subjects such as kinetic evidence of enzyme-substrate intermediates, pH dependencies, and so on, which can be related to the bond-forming and bond-breaking steps. It is unsettling to realise how so much sophisticated analysis can yield so little understanding of the forces being studied. In a review of Irwin H. Segal's *Enzyme Kinetics* (Wiley: New York, 1975), a text of similar scope, S.E. Halford (*Nature* **259**, 255; 1976) noted that enzyme kinetics is a tool of biochemical research, not an intellectual discipline in its own right. Even as a tool, Segal's *Enzyme Kinetics* is more detailed and more accessible than this newer volume.

The results of the efforts of legions of enzymologists are brought together in *Enzymatic Reaction Mechanisms* by Christopher Walsh. This text, as the author notes in his preface, developed from his

Atmospheric Physics

J. V. IRIBARNE and H.-R. CHO

1980, xii + 208 pp. + indexes
Cloth Dfl. 40,- / US \$ 15.95
ISBN 90-277-1033-3

Atmospheric Physics is an elementary and comprehensive textbook on the terrestrial atmosphere and can be used for second and third year university courses. The book requires only the basic understanding of mathematics and such knowledge of physics as can be acquired in most first year general physics courses. It can be used in two ways. Firstly, a general review of atmospheric physics is provided for students who work or plan to work in other fields (such as geophysics, geography, environmental sciences, space research), but are interested in acquiring general information. Secondly, it will serve as a general and elementary introduction for students who will later specialise in some area of atmospheric science. Each chapter is concluded with a list of questions and problems which will help the reader to attain a more detailed insight into the various subjects discussed.

Microphysics of Clouds and Precipitation

HANS R. PRUPPACHER and
JAMES D. KLETT

1978, xvi + 714 pp.
Paper Dfl. 40,- / US \$ 19.95
ISBN 90-277-1106-2
Cloth Dfl. 85,- / US \$ 44.75
ISBN 90-277-0515-1

'This book no doubt will become a landmark in the realm of cloud physics, not only as an advanced textbook but also as a valuable reference. Its pages contain about as complete an exposition of current knowledge of the subject as one would hope to find anywhere.'

Horace R. Byers, *Bulletin of the American Meteorology Society*

'The book, being a comprehensive, up-to-date description of the subject, is an excellent reference text for lecturers, students and researchers in atmospheric sciences, especially those interested in weather modification and air pollution.' Hans Mörth, *New Scientist*

'There is little doubt that this impressive book will be of central importance to cloud physicists and scientists working in related fields.'

J. Latham, *Nature*

'I strongly recommend the book for scientific libraries, serious cloud physics students, and other scientists who need to go in-depth into this subdiscipline.'

Doyle Sartor, *Physics Today*

D. Reidel Publishing Company

P.O. Box 17, 3300 AA Dordrecht, Holland
Lincoln Building, 160 Old Derby Street,
Hingham, MA 02043, U.S.A.

own teaching, and it owes much to a course taught by his mentors R.H. Abels and W.P. Jencks.

The approach taken is to classify and present enzyme reaction mechanisms according to their chemical types: (1) group transfer reactions; (2) oxidation-reduction reactions; (3) elimination, isomerisation and rearrangements of substrate skeletons; and (4) the making and breaking of carbon-carbon bonds. This approach to organisation makes eminently good sense as it facilitates understanding of the basic patterns and principles underlying the reactions.

In presenting data which support the various enzymatic mechanisms, Walsh brings to bear evidence obtained from a wide variety of methodologies, including kinetic analyses, isotope exchange studies, protein chemistry, and the results of X-ray crystallography. The fundamentals of enzyme kinetics are presented, but they are treated superficially and are interspersed with discussions of various enzymes.

The grand scope of this text results in, at times, a superficial coverage of material pertaining to many mechanisms. It would have been preferable to have seen fewer examples of enzyme mechanisms examined in greater depth. Nevertheless, this original, ambitious work fills a need, and it is likely to be welcomed by both teachers and students of enzymology.

Principles of Enzymatic Analysis edited

by H.U. Bergmeyer and K. Gawehn is directed not toward the classroom but toward the laboratory. In this book enzymatic analysis means determination of the concentrations of substances with the aid of enzymes and determination of the catalytic activities of enzymes in biological materials. It is derived from a section of a larger work, *Methods of Enzymatic Analysis* by the same editor (Verlag Chemie and Academic Press, 1974), which has already gone through two editions. This revised version is a modest advance over its predecessor.

In this volume 20 authors have contributed to sections describing theoretical principles (including kinetics), the handling of biochemical reagents and samples, measuring techniques and instruments, and evaluation and assessment of experimental results. The derivation, content and format of this book will probably restrict its use to that of a reference work. As such the prospective user should be advised that it is limited in scope and often overly brief. This book is not intended for someone who wants to learn how enzymes function, but it may serve as a practical handbook for someone who is about to do his first enzyme assay. □

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Biochemistry for all occasions

Ernst Noltmann

Biochemistry: The Chemical Reactions of Living Cells. By David E. Metzler. Pp.1129. (Academic: New York, 1977.) \$22.50.
Modern Concepts in Biochemistry. By Robert C. Bohinski. Pp.600. (Allyn and Bacon: Boston, Massachusetts, 1979.) \$17.50.
Biochemistry. By Frank B. Armstrong and Thomas P. Bennett. Pp.504. (Oxford University Press: New York, 1979.) \$19.95
Elementary Biochemistry, An Introduction to the Chemistry of Living Cells. By Julian Davies and Barbara Shaffer Littlewood. Pp.346. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$20.75.

WITH biochemistry having become a serious (?) topic of prime time television talk shows, including guest appearances by dignitaries such as Linus Pauling, it is not surprising that this popularity is also reflected in an annually increasing onslaught of new textbooks of bio-

chemistry which present a confusing choice to both the instructor and the student. A most telling testimony is perhaps also the fact that this reviewer was asked to render an opinion simultaneously on four different textbooks. An attempt will be made to comment on the four books in the order indicated above which is according to decreasing size but which also reflects an order of value as perceived by this reviewer.

Metzler's coverage of the field is current and thorough. It is supported by many good illustrations and by superb bibliographies for each of the sections. The emphasis appears to be fairly even and the decision of the author to include many fascinating applications of biochemistry in special boxes set off from the main text, rather than to incorporate them into the narrative flow of fundamental material, is of great help to the student whose academic programme may demand a judicious use of study time. The detail of Metzler's text is both an advantage and a disadvantage. It makes reading slow for the novice. However, the writing is good and the style interesting. Moreover, the sentences are varied and well constructed — something rare for a science text. Also, on re-reading, the detail does not appear to be excessive and the opportunity to pursue

details through the extensive, up-to-date bibliography makes the book a good reference source. Metzler's book is definitely designed for the serious biochemistry undergraduate major or graduate student. This reviewer would place it as a text besides or even ahead of such greats as "White, Handler and Smith", "Mahler and Cordes" or "Lehninger."

Bohinski's book is, in general, written in a clear fashion. Individual chapters, although beginning at the level of understanding for non-majors, proceed with great detail which sometimes overwhelms the student, who may have difficulty placing the information in the proper perspective. In our department, the text serves a one-quarter course for non-biochemistry science majors for which it was found to be a good background text because of its emphasis on structure-function relationships and on regulation as a key to understanding metabolism. It may be suitable also for a two-quarter course if students come with a solid background in biology and chemistry. Some definitions appear to be misleading. For example, this reviewer believes the explanation of quaternary structure (p.145) to be incorrect. The desire for completeness has led the author on occasion to use metabolic schemes too complicated to be didactically useful (for example, fatty acid synthesis). Some students feel that additional examples of problem-solving would be helpful for the understanding of pK_a 's, ΔE° , and the intricacies of peptide sequencing. Of all the four texts the print of Bohinski's book is the most difficult to read, especially when it appears in the italic mode. As a special point, this reviewer enjoyed the essay by Morowitz on the "Six Million Dollar Man" included in the Preface. It serves well to put it all in perspective.

The book by Armstrong and Bennett presents biochemistry at a level and with a degree of detail adequate for a service course for non-majors for whom biochemistry is more an ancillary requirement than a discipline of interest for its own sake. The coverage of the various biochemical topics varies and seems to be influenced by the preference of the authors. The chapter on biogeochemical cycles is unusual. The treatment of enzymes and nucleic acids is thorough. Repair and recombination of DNA are dealt with in a very up-to-date fashion. A separate chapter on nutrition is particularly valuable. In fact, it is most deplorable that this vital area of biochemistry is neglected by most textbook authors. Unfortunately, practically no mention at all is made of fatty acid synthesis, and synthesis, in general, is given little attention. Frequent reference is made to medical relevance which is usually of interest to students, including those who are not premeds. This reviewer liked the historical introduction as well as the

summaries after each chapter, the latter allowing students to place the various subfields of biochemistry in relation to each other.

Finally, *Elementary Biochemistry* by Davies and Littlewood is quite different from the other three books reviewed here. While the authors warn that the title may be misleading, this reviewer feels that the simplistic treatment may be just that, namely misleading. A treatment of enzyme kinetics without ever using the word "kinetics" or without mentioning Michaelis and Menten or Lineweaver and Burk appears to be less than "elementary". However, if perused only by those whose only aim is to get a "feeling" of biochemistry "for fun", it is a nice means to seduce perhaps the non-science

student to probe more deeply into what biochemistry really is. The descriptions of some biochemical techniques are very good and may induce those whose interests are aroused to look further. Interestingly, the problem sets provided by the author appear to be much more difficult than the text suggests. Students will need considerable help from the instructor and from teaching assistants in order to be able to solve them. Nevertheless, the book has a good format. It reads easily, the summaries are clear and, as a whole, the book may help newcomers to sense the excitement of "being in biochemistry". □

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Basic chemistry

James Viers

Fundamentals of Chemistry. By Fred H. Redmore. Pp. 711. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$18.95. *A Basic Math Approach to Concepts of Chemistry*. By Leo Michaels. Pp.312. (Brooks Cole: Monterey, 1979.) *Fundamentals of Chemistry*. By E. Kostiner and J. R. Pea. Pp.472. (Harcourt Brace Jovanovich: New York, 1979.)

IN a foreword to *Fundamentals of Chemistry*, by Fred H. Redmore, the statement is made that the text was developed out of an attempt to expand and upgrade *Basic Chemistry* by Seese and Daub into a general chemistry text. I do not feel that either the expansion or the upgrading is sufficient to place *Fundamentals of Chemistry* into the category of a desirable general chemistry text which will properly prepare students for higher level chemistry. While most of the topics normally found in general chemistry texts are included here, the treatment of a number of topics is somewhat cursory. The kinetics chapter, for example, totals 11 pages (as does the chapter on co-ordination compounds) including summary, exercises and problems and a page of objectives. Perhaps the biggest shortcoming in the book is the number and type of end-of-chapter problems. The chapter on kinetics mentioned previously has only two exercises (of a qualitative nature) and three rather trivial problems. Other chapters do contain more problems many of which have multiple parts. However, almost all of the problems are basically of the drill and practise type, with few challenging ones and very little application of chemical principles to relevant or practical types of problems. An instructor who uses this text will very likely be forced to use

supplementary problems extensively.

There are a large number of general chemistry texts on the market today which are superior to *Fundamentals of Chemistry* for use in the main line course which is structured to prepare science and engineering students for organic and/or physical chemistry. If this text has a market, it might be in the introductory chemistry course currently offered as a year-long terminal course at many schools. The book is well written and very attractively designed in a two-colour black and red format. It can be recommended for those courses which are intended to be terminal and for a course in which the instructor wishes to present chemistry in a rather qualitative fashion.

A Basic Math Approach to Concepts of Chemistry, by Leo Michaels, is a workbook, basically of the programmed type, in which each unit is broken into parts and frames. Questions within each frame are designed to enable the student to master each idea before moving on to the next frame. The purpose of the text is purportedly to teach students who are taking or preparing to take a college course in chemistry the mathematical skills necessary for such a course. Some of the mathematical topics covered, however, border on the absurd for any student in college. Unit 1, for example, deals with the naming of whole numbers (example: Write the number that goes with the word name eight thousand). Unit 2 treats naming, adding, subtracting, multiplying, and dividing of decimal numbers, while unit 3 covers the definition, adding, and subtracting of signed numbers. Unit 4 teaches the student to write numbers in power-of-ten form and to multiply and divide numbers in power-of-ten form. It is inconceivable that any college student who has graduated from high school or even elementary school should need to review mathematical material of this nature. The remainder of the 14 units are devoted to topics which are somewhat more advanced and more directly related to calculations of

a chemical nature. These topics, however, are covered at a rather superficial level. The only type of student who is likely to benefit from this workbook is a student whose high school mathematical training is virtually nonexistent. If the student really needs exposure to many of these topics, it is very doubtful that he will be able to compete successfully in a true college level general chemistry course.

Fundamentals of Chemistry by E. Kostiner and J. R. Rea, is designed for a one term, preprofessional introductory chemistry in a straightforward, easily readable style. The book is attractively designed in a two-colour black and red

format which makes it very easy to read. Major topics found in most full year texts which are not presented here include: kinetics, thermochemistry, nuclear chemistry, co-ordination compounds and a discussion of the transition elements. A reasonably large number of worked-out example problems are included in most of the chapters as well as a fair selection of end-of-chapter problems. Fairly extensive chapters on organic chemistry and biochemistry (about 13% of the text material covers organic and biochemistry) are attractive features of the book and should appeal to the audience for which the text is designed. Historical and relevant

sidelights are prominently displayed throughout the text and also add appeal. The book emphasises the basic concepts of chemistry, however, and is not of the 'cutesy' variety with cartoon-type drawings which has recently become popular for use in the abbreviated one term general chemistry course. This text is highly recommended for instructors who wish to present a one term course in general chemistry which emphasises the basics of chemistry. □

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Experimental organic chemistry

P.F. Schatz

Experimental Organic Chemistry. By David Todd. Pp.346. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$13.95. *Techniques and Experiments for Organic Chemistry*. Third edition. By Addison Ault. Pp.442. (Allyn and Bacon: Boston, 1979.) \$16.95.

RECENTLY, there has been a flood of organic chemistry laboratory manuals from the book publishers. Among these books are revised editions of previous manuals and a few brand new manuals. On perusing the manuals it is sometimes difficult to avoid a sense of *déjà vu*, for there is a great deal of overlap in the experiments detailed. One new book that does not suffer in this way is *Experimental Organic Chemistry* by David Todd. Although there are a few familiar faces, this collection of fifty-eight experiments contains many new experiments for the undergraduate. With this large number to choose from, an instructor should have no difficulty in selecting enough interesting and instructive experiments to fill an introductory organic laboratory course. Examples of common organic preparations, Grignard reaction, Friedel-Crafts acylation and Fischer esterification to mention a few, are included, as well as some not so ordinary preparations such as the Peckmann coumarin synthesis and the Rothmund synthesis for the preparation of tetraphenylporphyrin.

Unlike many laboratory manuals, which present laboratory techniques separate from the experiments, Todd introduces the techniques as an integral part of a specific experiment. For example, column chromatography is explained and

demonstrated in an experiment on extracting pigments from spinach. As a consequence of this approach, one could be locked into doing a certain core of experiments in order to introduce the basic techniques. The experiments are detailed enough to be carried out by the inexperienced undergraduate student. The discussions of the techniques are concise and give enough information for the student to understand the how and the why of the techniques. The section on NMR and IR spectroscopy is very short (7 pages) and just covers the basics. This would not be the book for a laboratory course that is heavy on instrumentation.

Among the good points of the manual are the detailed instructions conveyed in a very conversational style. This, coupled with a section entitled "Most Common Errors", which appears after each set of instructions, should lead to good results for most students. Another favourable point is that many of the experiments and compounds encountered in the experiments can be related to familiar, naturally occurring materials. The NMR and IR spectra of thirty-five compounds are illustrated, but they appear to be retracings of spectra as opposed to reproductions of actual spectra (the coupling in the methyl triplet of the ethanol spectrum on page 97 does not appear to be symmetric as it should be). Also, the solvents used to prepare the NMR samples are not noted on the spectra.

There is some inconsistency in the warnings regarding hazardous properties of the chemicals. For example, the toxicity of benzene is noted in Experiments 25 and 33, but no mention of it is made in an earlier one — Experiment 13, the Grignard synthesis of monodeuterated benzene. Also in Experiment 13, 1,2-dibromoethane is suggested as a reagent for starting reluctant Grignard reactions and no warning is given regarding its toxicity. This compound is on the OSHA tentative Category 1 carcinogen list, as is benzene. Thus, any instructor using this book, or any other laboratory manual for that

matter, should check to see if the hazard warnings included need to be supplemented or updated.

Among the revised editions of organic chemistry manuals is *Techniques and Experiments for Organic Chemistry* by Addison Ault. This is an updated and expanded version of a good laboratory manual. Better than one-half of the book is devoted to describing techniques and apparatus for the organic chemistry laboratory. This is done thoroughly and clearly and is illustrated with large, accurate drawings. Modern spectroscopic techniques, NMR, IR, UV and mass spectrometry, are dealt with extensively.

The second half of the book is a compendium of experiments demonstrating the techniques and the characteristic reactions of organic compounds. The experiments are divided into four groups: separations, transformations (one-step procedures), synthetic sequences and projects. The separations, transformations and synthetic sequences are all well worked out, carefully detailed experiments which provide good working experience with organic chemistry.

For the more adventurous and skilled, there are the projects. These are procedures excerpted directly from the primary sources in the chemical literature. The compounds included are natural products, are theoretically interesting, or have unusual physical properties. The ability to reproduce procedures directly from the chemical literature is very important to the organic chemist.

In summary, Ault's book is a well written modern, informative manual that should be looked at seriously by anybody who is considering using laboratory manuals. □

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Basic botany

Ralph S. Quatrano

Botany: A Brief Introduction to Plant Biology. By Thomas L. Rost, Michael G. Barbour, Robert M. Thornton, T. Elliot Weier and C. Ralph Stocking. Pp.344. (Wiley: New York and Chichester, UK, 1979.) \$20.15. *Plants: Basic Concepts in Botany.* By Watson M. Laetsch. Pp.510. (Little, Brown & Co.: Boston, Massachusetts, 1979.) \$17.95. *Instructor's Manual to Accompany Plants: Basic Concepts in Botany.* By Watson M. Laetsch. Prepared by Mary S. Manteuffel. Pp.121. (Little, Brown & Co.: Boston, Massachusetts, 1979.) \$17.95. *Instructor's Biology.* By Kingsley R. Stern. Pp.482. (Brown: Dubuque, Iowa, 1979.) \$13.95. *Laboratory Exercises/Biology of Plants.* Fourth edition. By H.L. Dean. Pp.326. (Brown: Dubuque, Iowa, 1978.) \$9.95. *The Green World, An Introduction to Plants and People.* By Richard Klein. Pp.512. (Harper and Row: New York, 1978.) \$14.95.

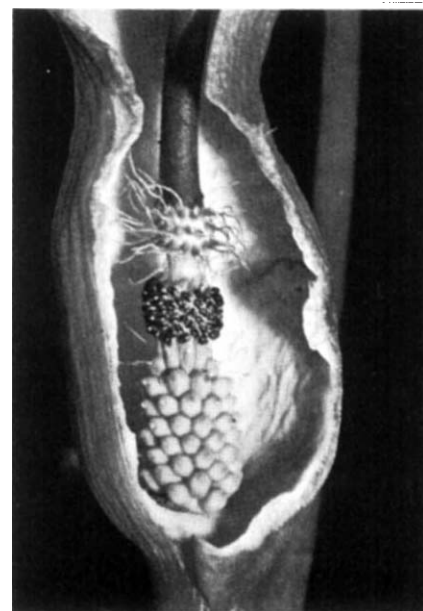
MOST recent textbooks of plant science that are aimed at beginning undergraduates can be grouped into two classes: those that approach the subject from a more traditional and classical manner, and those that popularise plants by emphasising their uses and values to society, that is, ethnobotany. The former tend to be used in courses for agriculture, forestry and biological science majors. Such courses usually serve as a technical framework upon which is based an advanced curriculum in morphology, physiology, ecology and systematics. The other texts began to appear during the last decade and tend to be used in non-major courses and in two-year colleges where exposure to plants, their diversity and value to society are stressed. The texts reviewed here represent a wide choice between these categories.

One of the best and more popular classical texts for majors by Weier *et al.*, *Botany*, has now been published in a shortened version, *Botany: A Brief Introduction to Plant Biology* by Rost *et al.* In many ways this text is a revision of the larger fifth edition (1974), since much of the subject matter has been rewritten and updated and new drawings and figures added. It is still a basic plant science text with no major change toward a more simplified or popularised treatment of the science. (Generally, the selection of topics to retain and condense was good, considering the difficult job of reducing a 642-page text to 344 pages (a slightly larger format, however).) The bacteria and virus chapters were dropped and extensive coverage of certain topics was reduced (for example, CAM metabolism and C_4 photo-

synthesis). However, all the basic botanical subjects are well represented in breadth and depth in the revision. The illustrations are excellent, especially the drawings. The basic organisation and groupings of topics are flexible; subject matter usually covered together can be assigned as units (chapters/appendices) rather than scattered pages. The one minor exception is the coverage of genetics where the molecular aspects of gene expression are covered within the chapter on metabolism separate from mendelian genetics. This is a well written text, with a good glossary, chapter summaries and a basic chemistry appendix. This text couples a sound treatment of the essential topics to be presented in an introductory plant science course with conciseness and brevity. It would be a good text in a short course for majors, the "plant" text in an Introductory Biology series and an alternative to a popularised treatment of plant biology for an introductory course at a junior or community college.

Laetsch has written a text for majors (*Plant: Basic Concepts in Botany*) that presents the traditional topics but in addition includes a considerable emphasis on the role, uses and importance of plants in the lives of students. His approach is to present topics in a context with which students are familiar and to stress the importance of plants in their lives. Energy is introduced in the first chapter within the broad framework of the biosphere which includes mention of food chains and energy transfer within ecosystems by use of common examples. This chapter and the following one entitled "Agricultural Revolutions" will impress on the reader the importance and usefulness of plants to their existence and the future of mankind. A fairly typical coverage of the plant kingdom follows in the second part of the text which exposes students to the diversity of plants. Taxonomic principles are covered using Angiosperms that everyone knows, that is, "the supermarket plants". This is extended in the Appendix (Supermarket Taxonomy) in which a brief taxonomic description is given for 16 selected families of popular dicots and monocots. The last chapter of this section deals with "People's Plants", in which 16 plants are presented in a historic and socio-economic framework to acquaint students with those "plants that keep our culture running" (wheat, sugar cane, rubber, coffee, tobacco).

These first two sections should serve to heighten the interest of the students to delve into the last two sections dealing with the structure/function and evolution of seed plants. Here the organisation of the material is similar to most other texts and will allow an instructor considerable flexibility in covering the basic and more popular topics in any sequence he desires and still be able to assign individual chapters. However, the depth in which most topics are covered (for example, photosynthesis/respiration and survey of



The chamber or spathe of the cuckoo pint traps flies carrying pollen from other plants, so aiding its fertilisation.

From *Color in Plants and Flowers* (Everest House: New York, 1978)

the plant kingdom) is not as extensive as in other texts for majors such as (*Biology of Plants* by Raven, Evert and Curtis). Little chemistry is included (no structural formulae) and hence the freshman with no chemistry background would have few problems with this text. The author has introduced basic botanical principles in a way that will stimulate the student's interest and excitement in the plant sciences. The text is well written and includes extensive references, chapter summaries and review questions, a glossary, as well as 1-2-page supplements scattered throughout the book. These add more depth and help explain abstract concepts that might interfere with the flow of the text. A helpful *Instructors Manual* is included. The text is different from others now on the market but in a way that does not eliminate its use in any traditional plant science course or in a non-major course. I suspect this text can be adopted by a wide range of existing courses at different types of educational institutions.

The text *Introductory Plant Biology* was written by Kingsley R. Stein to "emphasize current interests (of students) without giving short shrift to botanical principles". Stern's approach is that the basic science of botany is more meaningful to beginning students if its relationship to the more applied fields (for example, horticulture) is emphasised. This text, like the one by Laetsch, falls between the two extremes of introductory plant texts. For the most part, the integration of applied botany into the main part of the text does not constitute much more of an emphasis than most other traditional texts. However, 90 pages of Appendices represents most of the emphasis on practical botany. This includes a section on the use of biological controls as an alternative to chemical control by pesticides and herbicides, a

listing and brief description of useful plants (for example, wild edible plants, poisonous and medicinal plants), home gardening tips and information about conditions for optimal growth of common houseplants. Also included in the Appendices, however, is a section with more details of DNA structure and function, photosynthesis and respiration for those who wish a more in-depth treatment. The first 10 chapters of the text represent the areas of morphology, physiology, development and genetics while the remaining 11 chapters deal with classification, ecology and a survey of the plant kingdom including bacteria, blue-green algae and viruses. One of these chapters, entitled "Flowering Plants and Civilization", discusses 15 well known families and, using representative examples of each, Stern briefly describes their ties with various cultures as well as their economic importance.

Review and discussion questions follow each chapter as well as a glossary at the end of the text. A well illustrated and proven (fourth edition) lab manual is available from the same publishers (*Laboratory Exercises. Biology of Plants* by H.L. Dean). Although this book can be used in major courses, the depth and level of coverage of topics plus the nature of the Appendices suggest a probable wider acceptance in the non-major or non-science courses.

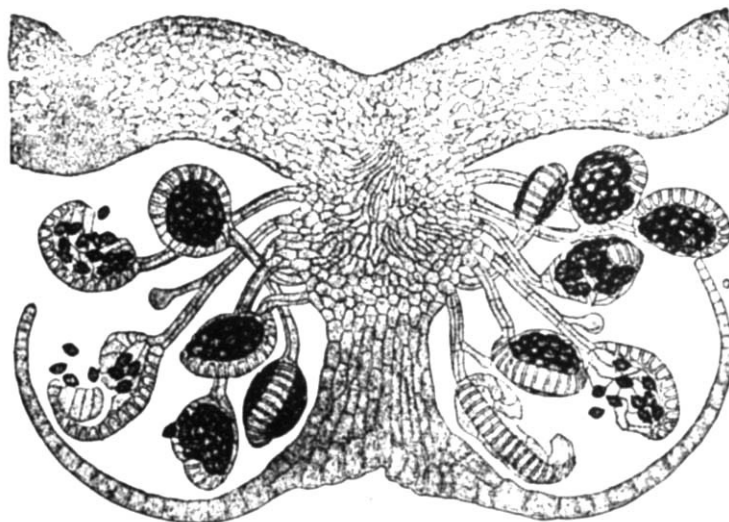
The *Green World. An Introduction to Plants and People* by Richard Klein is clearly different from the above texts. It is a unique contribution to botanical books and represents the opposite end of the spectrum from traditional, technical texts. His approach is clearly a historical one in presenting plants as an important component of various cultures. The author's view point is that "through an examination of the green world we can better understand the modern world and

the civilization from which western culture has evolved". With this objective his text is quite successful. He presents fascinating and exciting historical and cultural facts about plants that will no doubt interest any reader. Chapter titles include Economics and Politics of Food, Plants in Religion, Medicinal Plants, and subheadings such as Spices and Savory Herbs; Plants of Superstition, Myth and Ritual; Herbs, Herbals and Herbalists; and Botany of Coffee. It is not a popularised or strictly applied text but more of a scholarly, intellectual presentation of the role plants have played and will continue to play in society. It is flavoured with the personality of the author which is quite refreshing. However, the coverage of the topics normally found in the classical texts is brief and simplified and mainly confined to scattered one- or two-page essays or supplements to the major chapters. Conspicuously absent are coherent sections on morphology, mendelian and molecular genetics, survey

of the plant kingdom and respiration to name a few. With the exception of a few references at the end of chapters, there are none of the usual aids such as chapter summaries or review questions and a glossary. I doubt if this book will be used as the sole text in a beginning botany course for science majors. It would have merit as supplemental reading in such a course, in a non-major course, or as a text in a seminar course for advanced botany majors interested in a different and valuable perspective.

Instructors of plant science have quite a diversity of good new texts from which to choose the vehicle to convey the science and excitement of botany to young, eager minds. □

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Vertical section through the sorus of the fern, *Dryopteris*, showing sporangia in various stages of development.

From *Intermediate Botany* (Macmillan: London)

Modern virology

General Virology. Third edition. By S.E. Luria, James E. Darnell Jr, David Baltimore and Allen Campbell. Pp.578. (Wiley: New York and Chichester, UK.) \$23.50.

THIS excellent textbook uniquely fills a major need in the biological sciences: a single volume to introduce the many facets of modern virology. It is clearly written and well organised, so that it can be used essentially as the sole reference source for a general virology course.

As expected from the credentials of the authors, the book heavily emphasises the

P.C. Kimball

molecular level of knowledge about viruses. To take full advantage of this text, students should have had some previous exposure to the basics of molecular biology from a biochemistry or genetics course (or both). Certain chapters summarise the principal features of gene expression and animal cell biology; however, as indicated by the authors, these helpful reviews cannot substitute for a more thorough treatment of these topics prior to using the text. Some previous exposure to advanced mathematics, especially an introduction to statistical concepts, would also be useful to the students. Thus, this text is recommended primarily for advanced

undergraduates or graduate students majoring in the biological sciences. To read the entire text in one academic quarter will be an intense experience for such students; those in the semester system will still find the breadth and depth of material challenging. For these reasons this text is not recommended for an introductory course for less advanced undergraduates or those not majoring in a molecularly orientated programme; unfortunately, no text suitable for these latter students is presently available to provide the same broad coverage of the field on a more introductory level.

The book is well documented with an 80-page bibliography. Therefore, it will continue to serve the students as a detailed reference book. Although important new developments are frequent in virology in

this age, the text is thoughtfully written to prevent rapid obsolescence. For example, the book went to press just before the first convincing demonstrations that RNA splicing is required for production of mRNA in several mammalian systems. Nevertheless, the background for this finding is carefully developed in discussions on the relationship between cellular hnRNA and mRNA, and in the summaries of known details of viral mRNA production. In fact, the authors correctly predicted that the adenoviruses would be one of the most productive systems for clarifying precursor-product relationships in mammalian mRNA

synthesis. In general, where significant controversy exists, the authors present a balanced view and try to indicate the experimental approaches most likely to lead to resolution of the issue. Another strong point is the thorough description of most virological methodology in frequent use today.

The one major deficiency of this text for some purposes may be the relative lack of emphasis on the overall effects of viruses on the host, especially in higher organisms. Thus, the nature of viral diseases and defence mechanisms in animals are given only summary treatment, while there are only hints of the importance of plant and

insect pathogens for agriculture. Many students continuing their education in the biological sciences may encounter some of these topics in other specialised courses, depending on their interests; but some teachers may wish to supplement the text in these areas for the sake of providing greater appreciation of the more biological aspects of virology, to complement an otherwise complete introduction to the field. □

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Basic biology of malignancy

Joseph R. Bertino

Fundamentals of Oncology. By Henry C. Pitot. Pp.192. (Marcel Dekker: New York, 1978.) \$9.95.

THIS concise textbook, which stems from a course given at the McArdle Institute for Cancer Research at the University of Wisconsin, is directed toward non-physician scientists, undergraduate and graduate students, and postdoctoral fellows in the biomedical sciences. Actually, medical students would also find in the book a great amount of material not covered adequately in their courses in pathology and medicine.

Since the book is written by one author, the writing is consistent throughout, and the 12 chapters are all equally well done. The author writes in a lucid direct style, and is able to present the reader with the important aspects of each topic with sufficient definition and background so as to be easily digested. Each chapter is referenced; the student who wishes to read in more depth on a particular topic will find all of the key references. The titles of articles are included, so that although the text is not documented (which would diminish its readability) the appropriate references for each section can be found. The author is an experimental pathologist and biochemist, and I found the chapters on aetiology and pathogenesis of cancer, and the biochemistry of cancer especially well done. Since these chapters comprise most of the message, the book is very successful. Other textbooks may contain

more information on specific topics, but none present so much information in such a well written, concise fashion.

The book does not cover topics such as cancer diagnosis or cancer treatment (chemotherapy, surgery, immunotherapy and radiation therapy), but the student who masters the material in this book will have the background to understand these more clinical topics.

In summary, this book is highly recommended for both undergraduate or graduate students as well as physicians who wish to know more about the basic biology of malignancy. □

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History of biology

Dov Ospovat

A History of the Life Sciences. By Lois N. Magnier. Pp.489. (Marcel Dekker: New York, 1979.) \$23.50.

LOIS MAGNER'S *History of the Life Sciences* was written as a textbook for undergraduate courses in history of biology (it does not pretend to encompass the history of medicine). The discussion effectively begins with the pre-Socratic philosophers and continues into the "post Watson-Crick period" in the study of genetics. Some 70 pages are devoted to ancient and mediaeval science and another 40 pages to the Renaissance and Scientific Revolution. Most of the remaining three-quarters of the book treats the development of embryology, cell theory,

microbiology, physiology, and evolution from the seventeenth century to around 1900. Only in the last two chapters, both of which are concerned with genetics, is there any sustained discussion of twentieth-century biology. Scientific societies and microscopes are each accorded a brief chapter. There are some curious omissions. For instance, little attempt is made to relate seventeenth and eighteenth century biology to the Scientific Revolution. In the chapter on physiology Helmholtz is only mentioned, while Ludwig and Du Bois Reymond are completely ignored.

Magner's basic technique is as follows: under each chronological or subject heading, she discusses the individual scientists deemed to have contributed to the advance of biology. One result is that many key issues — such as the philosophical and religious concerns of seventeenth, eighteenth, and nineteenth century biologists, and the arguments amongst nineteenth century naturalists and physiologists over appropriate styles of

explanation — are inadequately treated, in a piecemeal fashion in the biographical sub-sections.

The biographical approach is no doubt convenient for author and students. Likewise, because it conforms to the pre-conceived notions of most undergraduates, presenting science as progressing toward the present is perhaps the easiest way to teach the history of science. But since it is now pretty generally agreed that this is a wholly unsatisfactory way of understanding the past, it is strange to see it adopted as the organising principle of a new textbook.

Magner often seems to be interested chiefly in whether a scientist was 'progressive' or 'retrogressive', as defined by mid-twentieth century knowledge and attitudes. Thus we are told that "although preformation theory impeded progress in embryology, it had one virtue. Preformationists generally rejected spontaneous generation" (page 188); that La Mettrie "paved the way for more

modern biological theories" (page 307); and that the *Origin of Species* was "a clear statement of the fact and mechanism of evolution and was quite convincing to readers with open minds" (page 385). Rather than attempting to present Haller's thought in its eighteenth century context, Magner praises him for his "restrained and modern spirit" (page 305).

The most serious weakness of the book is that Magner has made very little use of recent scholarship. As a sub-field of the history of science, the history of biology has come into its own in the last 20 years, yet Magner relies largely on much older works, predominantly books. Consequently, the presentation is often seriously deficient, given the current state of historical knowledge. This is especially true of the chapters on microbiology and biogenesis, evolution, and embryology.

I found numerous errors, some minor, others serious: the Reign of Terror is said to have occurred in 1789 (page 361); the relationship of von Baer's laws to the theory of recapitulation is mis-stated (page 204); the word 'biology' is said to have been coined as a replacement for 'natural philosophy' (page 405); and it is claimed that "taken as a whole" Buffon's writings "contain all the elements of the Darwinian synthesis" (page 358). □

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Historical and physical geology

Hermann W. Pfefferkorn

Earth History and Plate Tectonics: An Introduction to Historical Geology. Second edition. By Carl K. Seyfert and Leslie A. Sirkin. Pp.589. (Harper and Row: New York, 1979.) \$18.95. *Geological Evolution of North America.* Third edition. By Colin W. Stearn, Robert L. Carroll and Thomas Clark. Pp.556. (Wiley: New York and Chichester, UK, 1979.) \$25.90. *Interpreting the Earth.* By Robert R. Compton. Pp.554. (Harcourt, Brace Jovanovich: New York, 1977.) \$16.95. *Physical Geology.* By L. Don Leet, Sheldon Judson and Marvin Kauffman. Pp.490. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$24.25. *Physical Geology.* By Bob F. Mallory and David N. Cargo. Pp.538. (McGraw-Hill: New York, 1979.) \$16.95.

OUR understanding of the history of the Earth has changed dramatically with the appearance of plate tectonics as the unifying theory of geology. This change had to influence the teaching of historical geology profoundly. In pre-plate tectonic times historical geology was taught as a survey of local lithostratigraphies with endless lists of stratigraphic names, as a survey of the evolution of life, or ideally as a combination of both. The large number of lithostratigraphic names often made historical geology an exercise in memory. With the change in emphasis from acquisition of encyclopaedic knowledge to understanding geological concepts, many geology departments dropped undergraduate courses in historical geology. However, plate tectonics has given us a theoretical framework for the physical aspects of historical geology and simplified explanations for many geological phenomena. Thus, historical geology can now be taught with an emphasis on principle rather than detail. The first two books are examples of this new approach.

Seyfert and Sirkin present principles in the first seven chapters in 148 pages (evolution, geologic time, ancient environments, correlation and facies, plate tectonics). One chapter is devoted to cosmology and origin of the Earth. Chapters 8 to 14 (387 pages) deal with historical geology proper. Each of these chapters treats a large time interval like the Precambrian or late Palaeozoic. Only the last two chapters, on the Tertiary and Quaternary, are devoted to one period each. The Phanerozoic time scale and an extremely brief description of animal and plant groups are presented in Appendices A and B. Seyfert and Sirkin emphasise the history of North America but also treat the

rest of the world. Text figures are numerous and well selected. The maps of continent positions at different times are shown in an ingenious projection. Many maps and cross sections were apparently created or modified for this book and are easy to understand. The history of life is well integrated with the other aspects of historical geology.

Stearn, Carroll and Clark begin their book with a discussion of plate tectonics in Chapter 1 followed by seven chapters (147 pages) on principles (orogenesis, stratigraphy, correlation, time, cosmology, palaeomagnetism, evolution). The following chapters (377 pages) are grouped into four parts; the craton, the Cordillera, the Appalachians, and the Arctic and Cenozoic Ice Age. The discussion is restricted to North America and each part of the continent is taken as an example of one geotectonic province. For each area the geological history for the most significant time interval is given. Thus, some geological periods are treated more than once. An appendix gives concise descriptions of plant and animal groups. The book contains many excellent figures. I find it refreshing to see numerous figures from Canadian sources, which are not often used to illustrate textbooks. Graphs are well executed and two-colour printing improves clarity.

Both texts have about the same number of pages, use the same space for introductory chapters and main body, and are equally well suited to teach historical geology. Both make fascinating reading. The difference lies in their approach: time sequence with world-wide coverage (Seyfert and Sirkin) versus a case history of North American geological provinces (Stearn, Carroll and Clark). On the undergraduate level, historical geology has usually been taught as the second geology course after physical geology. The books discussed are written for just that sequence. However, while plate tectonics has unified many seemingly disparate aspects of historical geology, moving plates in ever changing configurations are a formidable matter for undergraduates to master so early in their geological education. Therefore, it might be more appropriate in the future to teach historical geology as a higher level course in the senior year. Both books could be used for such a course with some supplemental reading.

The other three books are all intended as an introduction to geology. They achieve this in different ways. Compton starts to analyse a certain area and to discuss the geological principles active there. The other two books follow a more classical approach. Leet, Judson and Kauffman begin with internal processes of the Earth and Mallory and Cargo with external ones. The surface processes are, of course, more familiar to the student and Mallory and Cargo have developed the most coherent sequence of chapters of the three books.

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Their book is further written in a language which is easy to read. All the figures are easy to understand and many of them are original. In summary, the text by Mallory and Cargo can be used well in a course with many non-majors. Leet, Judson and Kauffman's book is written for science majors and geology majors. It is well written, rather complete in its coverage and gives extremely instructive examples and case histories. The photographs of outcrops and landscapes are impressive. Graphs are easy to understand and well done. Compton integrates principles with the discussion of specific areas and examples of scientific studies. He begins with surface processes and situations which

have not only geological but also environmental significance. This is clearly the most original of the three textbooks discussed here. A course based on this book might motivate students of geology who might be turned off by other textbooks. The colour photographs of outcrops, rocks and minerals would be especially helpful in a course without a laboratory part. Nevertheless, the data presented are detailed enough for the highest level introductory course.

In each of these three books I found some graphs and explanations similar to the ones I use in teaching. Selecting one of the three as a textbook would be a difficult choice. The decision would partly be based

on the kind of students who would predominate in the class. The other consideration would be how much one would be willing to adapt the course to the textbook. Compton's book would be best suited if course and textbook would coincide in the sequence of their treatment of topics. Leet, Judson and Kauffman's book could most easily be used in a course with a totally different sequence of lectures. Mallory and Cargo's book stands between the two others in this respect. □

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Igneous petrology

S. A. Morse

The Interpretation of Igneous Rocks. By K. G. Cox, J. D. Bell and R. J. Pankhurst. Pp.450. (George Allen and Unwin: London, 1979.) Hardback £18; paperback £8.95.

AFTER half a century of near-starvation, students of igneous petrology are being presented with a succession of books that pay serious attention to phase diagrams. This book furnishes a square meal, devoting to phase diagrams more than thirty per cent of the text. And, joy of joys, at last we have a decent treatment of trace elements and isotopes, which occupy fifteen per cent of the text. The remaining half, liberally sprinkled with boldface where definitions occur, is taken up with such matters as intelligent classification, variation diagrams, fractionation, a respectable amount of petrography, experimental and controversial studies of natural rocks, volcanology, and plutonology (complete with flow equations). Appendices include directions for norm calculations and for plotting rock analyses in O'Hara diagrams. Exercises after each of the fifteen chapters are furnished with answers at the end of the book.

These blessings are not unmixed. Much space devoted to hypothetical phase diagrams could more usefully have been allotted to additional real ones with equivalent lessons as to their use. The quaternary projections may be overdone. The geometry of fractional melting is given short shrift, and its quantitative possibilities are mistakenly denied. Many readers will groan to find an unhappy treatment of variance which yields univariant points and divariant lines in 1-atm phase diagrams. Contrary to the

implications in the text, the isobaric restriction does rigorously reduce the variance by one, and this pitfall of nomenclature could have been avoided by use of an appropriate notation, such as F_p , to denote the restriction. Most of the classical phase diagrams are taken directly from the ancient literature without revision: we find albite melting variously at 1,120°C and 1,100°C rather than 1,118°C as determined by Greig and Barth in 1938. Kushiro's revision of Di-An-Ab is ignored except for being mysteriously ascribed to An-Ab. The incongruent melting of fayalite is not mentioned, nor is there any discussion of the role of oxygen in igneous fractionation. Carnegieite is said to "exsolve" on a reaction loop.

But one should not look a gift horse in the mouth. The text focuses on methodology and is offered to petrologists

of all ages. As an unintentional test of its utility, the review copy was thoroughly consulted, marked up and leaned upon during a recent seven-hour research conference, and it gave good service. The treatment of trace elements in the mantle and in magmas, and of radiogenic and stable isotopes, is a masterpiece of concise, clear erudition. Mantle isochrons and hydrothermal Taylor engines are not neglected. The text is plainly written throughout and easily accessible to students. In sum, this book is a most refreshingly welcome and, indeed, indispensable addition to the still pitifully small genre. □

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Chestnuts of the '40s

Brian J. Skinner

Exploration and Mining Geology. By William C. Peters. Pp.696. (Wiley: New York, 1978.) \$26.50. *Economic Mineral Deposits.* Third edition. By Mead L. Jensen and Alan M. Bateman. Pp.593. (Wiley: New York, 1979.) \$28.75.

THE generation of mining and exploration geologists trained during the 1950s had the delightful experience of reading H.E. McKinstry's eloquent volume *Mining Geology* (Prentice-Hall: Englewood Cliffs, New Jersey, 1948). There were no successors to inspire students of the '60s and '70s, but it is probable that William Peters' new book *Exploration and Mining Geology* will fill the role for the generation of the '80s. The need for a successor to

McKinstry's volume has become acute; the profession has grown enormously as mineral production has increased; mining methods have become more complex; many new techniques of geochemistry and geophysics have been added to the modern prospector's arsenal; the sophistication of geological information has grown dramatically and the economic conditions of today are far removed from those of the 1940s when McKinstry wrote his book.

Peters does not share McKinstry's mastery of English and his wry wit but he has prepared an admirable volume that the advanced student will find both pleasant and informative to read and that will also serve as a reference for the practising geologist. Throughout, he emphasises the observational and practical while at the same time urging the reader to keep an open mind. With good reason; new ways of looking at old ideas have found a lot of ore. The heart of the book, and the place where Peters' extensive experience shines through, is a nine-chapter section discussing what mining and exploration

geologists do and how they go about their business. But there are also concise and authoritative discussions of the origins and distribution of mineral deposits, of the engineering and economic aspects of mining plus descriptions of the way exploration programmes are organised and prospects evaluated. The volume is a mine of information and a pleasure to read.

How appropriate it would be to measure equal enthusiasm for the latest edition of another chestnut of the 1940s — Bateman's *Economic Mineral Deposits* — the book so many of us studied before we read McKinstry. When Bateman died in 1971, he had already started revising the volume. Perhaps he had delayed so long because the task of a total revision was beyond him; possibly he realised that the format of the volume, which dated back to the teaching pattern of his mentor, J.D. Irving, had become too cumbersome. Whatever the reason, when he did realise the task was beyond him, he asked his friend and protégé M.L. Jensen, formerly at Yale, now at the University of Utah, to complete the task. It was, in reality, a request to fit an old but successful format to a very different and rapidly growing field.

The experiment has not been entirely successful. The earlier editions were successful because they were a mass of reliable and carefully checked observations. When complex ideas were included (rarely) they were carefully explained. The present edition has the same broad coverage, the same mass of data and observations (some unchanged from earlier editions), but it is no longer reliable. Mistakes appear on many pages, especially where new material has been added. Many of these errors are minor to be sure, but they are, nevertheless, misleading and greatly reduce the value of the book for the beginning student. For example, the caption for Fig. 15-2 (a *P-T* diagram for the polymorphs of Al_2SiO_5) mentions "phase boundaries indicated by short dashes" as being not experimentally determined, and "longer dashes" being an extrapolation of the kyanite-sillimanite curve. But all boundaries shown are solid lines and in addition the reference used (Morey, 1964) is hardly the most recent or most authoritative. Even if mistakes are corrected in later printings though, the book introduces so many ideas, concepts and ways of plotting data without adequate explanation, that it is no longer the easily accessible volume that earlier editions were. The volume can probably be used as a class text (and presumably will be, because it has a broader coverage of topics than any other English language introductory text), but it will require very careful checking by the instructor and a lot of back-up information. □

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Instrumental analysis

J.F. Coetzee

Instrumental Analysis. By Henry H. Bauer, Gary D. Christian and James E. O'Reilly. Pp.832. (Allyn and Bacon: Boston, Massachusetts, 1978.) \$23.95.

THE explosive growth of chemistry is making it increasingly difficult to decide which material should be included in the curriculum. It is also making it increasingly difficult for one or two authors to produce a uniformly authoritative textbook, particularly at the higher levels. This is certainly true of textbooks on instrumental analysis, because instrumentation is developing at an ever-increasing rate and this is occurring in a variety of essentially unrelated areas. The answer is to employ as many authors as are necessary to ensure authoritative treatment of all topics included. Any unnecessary proliferation of authors should be avoided, however, because it would create unnecessary editorial problems in achieving the reasonable consistency in approach, emphasis, style and other matters required for a pedagogically effective textbook. The compromise reached in the present text many not be ideal in that the 24 chapters have been contributed by 26 authors. Six chapters have two authors each, and only three chapters have been written by authors who have also contributed another chapter. Considering this plethora of authors, the undoubtedly odious editorial task has been performed well in most cases, however. Two introductory chapters, one to electrochemical methods and another to

spectroscopic methods, contribute significantly to the effectiveness of the text; a third, on separation theory, would have been a useful addition.

The balance of topics included in a textbook such as this must reflect to some extent the personal bias of the editors, but this reviewer finds it entirely reasonable. The three main subdivisions of instrumental analysis — spectroscopic, electrochemical and separation methods — are treated in approximately 350, 140 and 110 pages, respectively. Other topics make up the remaining 200 pages; among these are particularly useful chapters on computers and automation. An additional chapter, dealing with a critical comparison of trace analysis techniques, would have been welcome. The emphasis throughout is on instrumental methods of quantitative analysis, rather than on electronics and instrument design. No laboratory exercises are included. The rationale for these editorial decision to make the theoretical background of each method "as brief and qualitative as possible consistent with clarity and accuracy," but instructors who wish to strengthen these parts of the text can easily do so. An adequate number of questions and problems are included with the majority of chapters, but future editions of the text would benefit from the inclusion of a few more truly challenging problems and, of course, correction of some errors in the answers provided.

In summary, this text has major strengths and only comparatively minor weaknesses. It represents a new and basically sound departure in producing a textbook on instrumental methods of analysis, and it should be on the short list of anyone considering such a text. □

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General chemistry

Jerry A. Bell

Chemistry: An Introduction. Second edition. By Sydney B. Newell. Pp.563. (Little, Brown and Co.: Boston, 1980.) \$15.95. *Problem Exercises for General Chemistry.* By G.G. Long and F.C. Hentz Jr. Pp.364. (Wiley: New York, 1978.) \$9.80.

THIS is an outstanding, short text that fulfills the author's purpose, "to provide beginning students and their instructors with a comprehensive, understandable, and motivating introduction to chemistry."

The book begins with a simple, descriptive picture of the atom, to make the four succeeding chapters on stoichiometry,

naming and equations, comprehensible. It goes on to atomic structure, periodicity and chemical bonding in more detail as the background for material on states of matter and chemical change (solutions, equilibria, electrochemistry and rates) as well as brief introductions to nuclear, organic and biochemistry.

Although mathematics is used throughout, the text is suitable for students that have difficulty with quantitative reasoning; most concepts are introduced by homely and timely examples that involve relatively little mathematics and jargon, there are exceptionally clear appendices on working with numbers and units, and all examples are worked out in

detail (by the factor-label method). Mathematics is treated as a tool and use of calculators is encouraged, especially when working with logarithms (pH). The end-of-chapter problems are grouped into two, roughly parallel sets and the solutions (set-ups shown) for the first set are given in an appendix; the rest of the solutions and suggestions for further problems, demonstrations, audiovisual aids, and so on are provided in an instructor's manual.

New terms are emphasised by italics and marginal notes in a second colour when they first appear; a very thorough index with a key to defined terms makes them easy to find later. Another feature is the appearance of a cartoon character, Maxwell's demon, in the figures. The demon is the author's "alter ego" and allows her to point out important features visually and informally, as many of us would like to do, if we could be present at our students' study. The demon is not gimmick, but an effective pedagogical device to which many students respond very favourably. Newell begins her first edition with the words, "I enjoy chemistry": students and instructors using her text will also.

The difficulties students have in solving problems generally fall into two categories:

interpretation and set-up. The second text is a self-study book that will give little help in interpretation.

The topics covered (in about 1,000 problems with answers) are the usual material of general chemistry: mole concept, stoichiometry, periodicity, atomic and molecular structure, phase properties, equilibria, thermodynamics, solutions, kinetics, and a bit of specific chemistry. (The material has also been used successfully in good high school classes.)

The authors' aim is to provide students with practice in working multiple choice problems to help them bridge the gap between the usual textbook problem statements and those they often meet in examinations. As the format and/or units of the answers are given in multiple choice problems, the student knows what she/he is working toward and is left 'only' the task of combining the information given in an appropriate way to get such a result.

The factor-label method is explained in concise, abstract form in Chapter 1 and then used consistently throughout the worked examples at the beginning of each chapter. Students who have trouble handling algebraic concepts will find the examples difficult to understand. Estimation (order of magnitude

calculation) of numerical results is emphasised as an aid to checking exact calculation and deciding whether a result is 'realistic'; often such an estimate is all that is required to select the correct response.

Occasionally, a bit more detail about the estimation made in the worked example would be welcome. The exact numerical solutions to the examples show what a student will actually see on a calculator followed by a discussion of precision, rounding-off, and significant figures; this approach is a very positive feature of the examples used. Significant figures to be reported in calculated values are explained both in terms of precision of the data and the more rote digit-counting procedure. The distinction between 'measured' and 'defined' values is made clearly in an appendix and applied throughout the text. The problems are straightforward, familiar and dull, that is, they provide no intrinsic stimulus for learning the material. Perhaps this is as it should be; this is a good practise book. The stimulus to practise must be provided by a teacher. □

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Analytical chemistry

M.S. Greenberg

Analytical Chemistry. Second edition. By Donald J. Pietrzyk and Clyde W. Frank. Pp.700. (Academic: New York and London, 1979.) \$17.50. *Quantitative Analytical Chemistry*. Fourth edition. By James S. Fritz and George H. Schenk. Pp.661. (Allyn and Bacon: Boston, Massachusetts, 1979.) \$19.95.

ANALYTICAL CHEMISTRY by D.J. Pietrzyk and C.W. Frank is a revision of an earlier text and is intended for introductory courses in analytical chemistry, especially those shorter courses servicing chemistry majors and life-science majors. Other texts with this objective dilute the fundamental analytical chemistry to render the text 'relevant' by emphasising analytical applications. By concentrating on fundamental principles of modern chemical analysis and illustrating these principles with applications to chemical, environmental, clinical and pharmaceutical problems, the authors provide the student, with some exceptions, with an excellent introduction to the theory and practice of analytical chemistry.

After a core of six chapters dealing with

prerequisite concepts, the text emphasises five major areas: volumetric methods, potentiometry, spectroscopy, chromatography and electrolysis methods. These chapters are followed by a chapter on experimental techniques and 41 laboratory experiments.

The core chapters include discussions of the nature of analytical chemistry, the analytical method, stoichiometry, statistics, operations in analytical chemistry and chemical reactions. These chapters are well written and very informative about the nature and role of analytical chemistry. However, some important concepts are not pursued in reasonable detail. The statistics chapter introduces the t statistic and confidence limits, but does not demonstrate their use in significance testing. Pronouncing that "the activity concept is an insignificant one" is bothersome. The introductory student should be aware of thermodynamic versus conditional equilibrium constants. Later, the authors note that since ion-selective electrodes respond to ion activity, ionic strength must be held constant to relate potential to concentration. However, the text does not demonstrate the calculation of or relationship between ionic strength and the activity coefficient.

The chapters dealing with volumetric methods of analysis are the best in the text. The authors show the student a chemical approach rather than a formula approach to problem solving — important equilibria are identified, equilibrium constants

written, chemically reasonable, simplifying assumptions are made and the problem solved. Further, the order of the chapters lends clarity to the material. For example, precipitation methods are divided into chapters on gravimetric methods and precipitation titrations, with the latter following the chapter on potentiometry. The chapters on redox methods and ion-selective electrodes are preceded by an excellent chapter discussing conventional cell notation and the Nernst equation. Tables summarising species determined and reagents and conditions for analysis by each method are also presented.

The remaining chapters on instrumental methods of analysis are all descriptive and contemporary. However, the emphasis on fundamentals, so evident in the earlier parts of the text, is missing. Deviations from Beer's law, their sources, elimination and implication in instrument design are not discussed. Instrument components and configuration (single beam, double beam) are not pursued with enthusiasm. On the other hand, differences in configuration for molecular versus atomic absorption instruments are pointed out. Chromatographic methods are developed without discussions of plate and/or rate theory and the Van Deemter equation. Electrolytic methods are introduced but no mention is made of the important new voltammetric methods.

The experiments are those which are done in most courses in analytical

chemistry: analysis of well characterised unknowns. The experiments are presented in a cookbook style, so the instructor may wish to supplement with assigned reading or lectures.

Despite the descriptive nature of the instrumental analysis chapters, this text is one of the best for teaching introductory analytical chemistry. The instructor may wish to supplement the instrumental analysis chapters with more theoretical concepts as noted, but the lucid writing and excellent organisation of this text teach the student to think analytically.

In revising the third edition, the authors of *Quantitative Analytical Chemistry* attempted not only to update the text, but also to make explanations clearer and difficult concepts more understandable. This revision is a significant improvement, and should maintain the popularity of the earlier texts in this series.

The text is intended both for short, introductory courses for chemistry majors and students in related disciplines and for more intensive courses for chemistry majors. The text is arranged in two sections: Part I, Principles and Theory, and Part II, Laboratory Techniques and Procedures. The authors recommend the first 14 chapters for a short course stressing the fundamentals of analytical chemistry, with the more specialised, 11 remaining chapters added for a more modern course in chemical analysis.

As a result of the dual audience objective, the chapter organisation in Part I is not ideal. After some introductory chapters, most texts present discussions of volumetric methods of analysis followed by chapters on instrumental methods. After the expected chapters on the nature of analytical chemistry, the analytical method and statistics, two chapters on gravimetric and spectrophotometric methods are presented. Overview chapters on volumetric methods and chemical equilibria follow immediately, then the theory and applications of aqueous and non-aqueous acid-base, precipitation, complexation and oxidation-reduction methods are presented in seven chapters. The instructor may wish to rearrange the middle chapters, especially moving the spectroscopy chapter to the end. The order of the more advanced chapters on instrumental methods is traditional.

As noted, the revised and new material have significantly improved the text. The statistics chapter is very good, yet a discussion of the use of confidence limits for significance testing would be expected for a majors text. The addition of worked problems in complexation chemistry lends clarity to that discussion. Chapter 5, Spectrophotometric Methods of Analysis, presents an excellent introductory discussion of Beer's law and chemical instrumentation. The best chapters of the text are those dealing with instrumental methods. The reorganised discussion of electroanalytical methods is followed by an

excellent chapter on potentiometry with ion-selective electrodes. As promised in the preface, the chromatography unit is strong, probably the best available in an introductory text. The chapters on atomic and molecular spectroscopy emphasise fundamental principles of spectroscopy and instrumentation, building on concepts presented in Chapter 5.

However, the "new treatment" applied to the presentation of acid-base chemistry was most disappointing. Approximations employed for pH calculations of weak acids and bases are not carefully justified. Although weak acids do ionise to a small degree such that the analytical and equilibrium concentrations of protonated acid will be nearly equal, the assumption is not justified or checked after the calculation. Further, implications of ignoring the autoprotolysis of water are not explored. For diprotic acids, the text states that if $K_{A1} \gg 100 K_{A2}$, the second dissociation may be ignored in pH calculations. Though this approximation is reasonable, it is not carefully justified. While the authors' intention to approach pH calculations by identifying the principal equilibrium and rearranging the

appropriate equilibrium constants is desirable, their presentation is not sufficient. On the other hand, their discussions on feasibility of titrations, indicators and the new chapter on non-aqueous acid-base titrations are good.

Part II is an acceptable laboratory manual. Experiments are grouped into units on analytical operations, gravimetric procedures, volumetric glassware, titrimetric, spectrophotometric, electro-analytical and separation procedures. Each experiment begins with a brief theoretical discussion followed by the procedure and concludes with questions. The experiments are those which characterise most teaching laboratories.

This text, though awkward in chapter progression, will function well in a chemistry majors course in modern chemical analysis. While it would be possible to use this text in a short introductory course, texts written specifically for those courses should be considered also. □

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General chemistry texts

R.O. Ragsdale

General Chemistry/Principles and Structure. Second edition. By James Brady and Gerard Humiston. Pp.786. (Wiley: New York and Chichester, UK, 1978.) \$19.95. *Laboratory Manual for General Chemistry Principles and Structure*. By Jo Beran and James Brady. Pp.520. (Wiley: New York and Chichester, UK, 1978.) \$8.95. *Basic College Chemistry*. By Don Roach and Edmund Leddy Jr. Pp.636. (McGraw-Hill: New York, 1979.) \$16.95.

THE text *General Chemistry, Principles and Structure*, by J.E. Brady and G.E. Humiston is a successful entry in a highly competitive market. Joint efforts between authors and publishers have resulted in a number of excellent texts at the level of Brady and Humiston. Some of them are: *A Conceptual Approach, Chemistry* by C.E. Mortimer (Van Nostrand: New York and London, 1979), *Chemistry, the Central Science* by T.L. Brown and H.E. Le May Jr (Prentice-Hall, Englewood Cliffs, New Jersey, 1977), and *Chemical Principles* by W.L. Masterton and E.J. Slowinski (W.B. Saunders: London and Philadelphia, 1977).

The Brady and Humiston text stands out alone with its unique feature of stereo

illustrations. The stereodiagrams as well as the other illustrations are all well done. I have been successful in seeing all of the stereodiagrams in 3-D. All of the stereo-illustrations are not necessary and in many cases students would be better off making their own models.

The text is well organised and well written. It has ample questions and problems. The second chapter, which introduces stoichiometry, has 19 examples and 63 questions and problems at the end of the chapter. The chapter on acid-base equilibria in aqueous solution has 15 examples and 71 questions and problems.

This is a traditional freshman general chemistry text for science and engineering majors. It is intended for both students who have had high school chemistry as well as those who have not. This text is similar to those mentioned above in using a theory and principles approach to the teaching of chemistry. Like the other texts it has a limited amount of descriptive chemistry. It has three chapters on representative metals, nonmetals and metalloids, and transition elements.

The freshman students in the US are probably at the same level as the advanced level students in the UK. The UK students are exposed to much more organic chemistry than their US counterparts. There has been a resistance to texts which offer much more than one chapter of organic chemistry. This is a paradox since many of these students will not be taking any more chemistry. This resistance to organic is also true for descriptive chemistry.

The Brady and Humiston text is very readable and the second edition seems to be an improvement over the successful first edition. Textbook authors are slowly going to the SI units. This text makes use of both SI units and the metric system. It will probably be some time before there is complete conversion to the SI system.

Most successful US books now come with a complete teaching package and Brady and Humiston is no exception. There is a student study guide available as well as a laboratory manual. The book, *Laboratory Manual for General Chemistry Principles and Structure* by J.A. Beran and J.E. Brady, has been designed to accompany the textbook. This manual is also suitable for a number of general chemistry texts.

The laboratory manual contains both quantitative and qualitative experiments (altogether 44 experiments). The emphasis on organic chemistry in the US general chemistry is reflected in the laboratory manual as 2 out of 44 experiments involve organic chemistry. There is a lack of emphasis on accuracy and precision in measurement in the manual.

In the first part of the manual 18 laboratory techniques are illustrated by actual photographs whose reproduction in the text are of high quality. For beginning students these instructions will be invaluable. The authors do have an

annoying habit of continually referring to these techniques. One would hope that the students would be able to refer back to the techniques themselves instead of the cookbook instructions (. . . 'remove copper from the rubber policeman . . . (Technique 6)' and ' . . . heat the copper metal to dryness with a Bunsen burner (Technique 9(a))'. The experiments all have a report sheet with questions and a pre-laboratory assignment sheet with questions and problems.

There are some interesting experiments. The law of multiple proportions is demonstrated by starting with copper (II) bromide and converting it to copper (I) bromide. Copper is obtained by displacement with magnesium. A limiting reactant experiment is achieved by taking an unknown mixture of the salts $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ and $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and adding water to them. Barium phosphate precipitates and one determines the limiting reactant and the composition of the salt mixture.

The third book that I reviewed left me in a quandary as to the students for whom it was intended. The text, *Basic Chemistry* by D. Roach and E. Leddy Jr, has a table of contents which is similar to texts that are used for science major courses. The authors give no indication in the preface for whom it was written. After perusal of the text, I came to the conclusion that it was

written for nurses and students in the allied health fields as the text is definitely a full level below Brady and Humiston. Some texts which are similar in level are *Foundations of College Chemistry* by Hein (Dickenson: Encino, California, 1977), *Basic College Chemistry* by Mitchell (Harper and Row: New York, 1978) and *Introduction to Chemistry* by Dickson (Wiley: New York and Chichester, UK, 1979). Roach and Leddy's text is longer than the ones mentioned above by some 100 pages. Many nurses' courses do general chemistry for one semester and in some cases only one quarter. The rest of the year is spent on organic and biochemistry. If the book is intended for this audience it has perhaps missed the mark.

It is conceivable that junior college and community college students might spend a year on a low level course. If these students then decided to go along the science major route they would need to take a Brady and Humiston type course.

Roach and Leddy's book uses a definition approach to chemistry. This approach makes the text seem quite sterile. The more difficult problems are starred. One wonders when a problem such as '4.21 g/ml = ? g/liter' is starred. □

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Soil chemistry

P.H. Nye

Soil Chemistry. A. Basic Elements. Edited by G.H. Bolt and M.H.M. Bruggenwert. Pp.281. (Elsevier: Amsterdam, Oxford and New York, 1978.) \$19.95. *B. Physico-Chemical Models.* Edited by G.H. Bolt. Pp.479. (Elsevier: Amsterdam, Oxford and New York, 1979.) \$73.25.

THESE two volumes have been largely written by Professor Bolt and his colleagues at the Agricultural University of Wageningen, The Netherlands. Part A (*Basic Elements*) is intended as a textbook for advanced undergraduates. Part B (*Physico-Chemical Models*) elaborates the theoretical material of Part A. Both parts concentrate on physicochemical aspects, and a general title such as "Soil Physical Chemistry" would have been more appropriate than *Soil Chemistry*.

The strength of Part A lies in its sound theoretical development, its sensible ordering of topics, its uniform symbolism and terminology in spite of nine authors, and the numerical examples at the ends of the chapters. There is no other textbook

covering the same ground at an advanced level.

The first chapter on the composition of the soil, and the second, a potted account of chemical, especially solubility, equilibria summarise the background anyone who hopes to understand the book must have. A fairly intelligible account of double layer theory and cation adsorption and exchange follows. The chapter on solubility equilibria in soils, including redox reactions involving iron, which are treated in terms of electron activity pe , is good. Chapter 7, "Transport and Accumulation of Soluble Soil Components", is often obscure (figure 7.1, for example, and the phrase "autonomous flux of solute"), though the distinction between self-sharpening and diffuse boundaries in solute displacement is well presented. These theoretical treatments are then used by Van Beeman and Brinkman to discuss soil formation in a way that achieves the right balance between ideal equilibrium and real non-equilibrium behaviour. The application of theory to saline and sodic soils is well presented. The volume concludes with an interesting account of soil pollution, which contains much more descriptive material than elsewhere in the book. We learn, for instance, that 60–90% of the deaths of street trees in Dutch town centres are caused by gas leaks following conversion to

natural gas. This section should convince students that the theory they have been invited to master in previous chapters is essential to understanding the fate of the great variety of pollutants now being dumped on the soil.

This second edition has eliminated many of the errors and 'Dutchisms' that occurred in the first edition. Unfortunately, the term "Free Enthalpy" is perversely retained throughout for Gibbs free energy G , without explanation. The uncertain student, having learnt to make this mental switch, will be mystified to read on p.14 that "to each chemical species in a reaction mixture a certain amount of ('free') energy can be ascribed." The phraseology is often awkward and some curiosities remain, such as a "gift" of irrigation water. It is a pity that the opportunity was not taken to have the text revised by an English writer.

Part B is considerably more difficult than Part A. The first half attempts to explain the relation between the proportions of exchangeable cations adsorbed on negatively charged clay and soil surfaces and their proportions in an equilibrium solution. Chapters 1, 2 and 3 by Bolt develop from first principles the theory of the diffuse double layer, the thermodynamics of cation exchange and the distribution of cations of differing charge in the electric field created by uniformly charged negative surfaces.

Much of this is Professor Bolt's own contribution to the subject, and it is good to have it collected together. The theory developed has limited application because it assumes the surface charge is uniform. In reality, the negative charges are discrete, so it is gratifying that Chapter 4 by Harmsen deals specifically with discrete site models, leading to a full account of the application of Guggenheim's theory of mixing to cation exchange equations. Diffuse double layer theory does not enable one to decide whether the selectivity coefficient in a heterovalent exchange (for example, $K^+ - Ca^{2+}$) is more likely to be constant if the activity of an adsorbed cation is represented by its equivalent fraction (Gaines and Thomas equation) or its mole fraction (Vanselow equation). The conclusion reached from mixing theory is that the selectivity coefficient should always lie between these two extremes. Up to this point Chapter 4 is well worth the effort; it then gets lost in remote realms of speculation, assuming various arrangements of discrete sites.

We are brought back to earth in Chapter 5. Bruggenwert and Kamphorst tabulate in uniform fashion thermodynamic data on 350 cation exchange reactions on clays, 265 on soils and a further 119 for heavy metals on soils, the last providing useful evidence of the high affinity of soil for these elements at low surface coverage; otherwise, they are able to draw disappointingly few conclusions from their mammoth collection of data.

In Chapter 6, Maes and Cremers consider the effect of surface charge density on ion selectivity in clays. In the exchange between mono- and divalent

ions, they show that a simple electrostatic model is only partially successful because of ion-solvent interactions that are difficult to calculate. The corresponding discussion of exchange between homovalent ions (sections 6.1.3 and 6.1.4) is marred by obscure and slipshod writing, and the accompanying tables (numbers 6.3 and 6.4) omit to mention the ions for which data are given. The greatly increased stability of ion complexes, such as copper-ethylenediamine, when adsorbed on clay surfaces is clearly described.

In a valuable account of anion exclusion from negatively charged surfaces, Bolt and de Haan suggest that the apparent reduction of chloride exclusion from the surface of calcium montmorillonite is caused by plate condensation rather than ion pair formation as suggested by Edwards, Posner and Quirk. This controversy badly needs settling by further experimental work.

A highly compressed chapter on the interaction of orthophosphate ions with soil lacks critical insight into the complex reactions described. If adsorbed anions were not to be more comprehensively covered — and phosphate is the only anion considered — this chapter might have been omitted.

The last third of the book deals with kinetics. Bolt treats movement of electrolytes in artificially packed soil columns in terms of advanced chromatography theory, and the few who will stay the course will be rewarded by a "guideline for guessing" the development of displacement fronts at the end of the chapter. Van Genuchten and Clearey extend this treatment to undisturbed

profiles, and show lucidly that a model based on zones of mobile and immobile water has marked success. Bolt and Groonevelt's chapter (Chapter 11) on electrokinetic phenomena in soil is the best account I have read and is neatly unified in terms of the thermodynamics of irreversible processes. The concluding section on the conductivity of clay suspensions reveals that exchangeable ions on clays have considerable surface mobility (p. 422), yet the treatment of diffusion effects in soil columns (p. 299) implicitly assumes that exchangeable ions are immobile. This inconsistency is not discussed. In fact, exchangeable ions in soils do have very different mobilities from exchangeable ions in clays, and the reasons for this important difference should have been referred to.

The book concludes with a suggestive account by Brinkman of clay transformations, in which the equilibrium and kinetic ideas developed in earlier chapters are applied to the long-term processes of soil formation. An advanced text on soil development from this viewpoint is badly needed and since good field men with an equally good understanding of physical chemistry are rare, perhaps Brinkman could be encouraged to write it.

This book should certainly be acquired by soil science libraries, and by wealthy workers in the fields of ion exchange and solute movement in porous media. □

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How to know

Almut G. Jones

How to Know the Grasses. Third edition. By Richard W. Pohl. Pp.200. (Brown: Dubuque, Iowa, 1978.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Mosses and Liverworts.* Second edition. By Henry S. Conard and Paul L. Redfearn Jr. Pp.302. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Lichens.* Second edition. By Mason E. Hale. Pp.246. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Seed Plants.* By Arthur Cronquist. Pp.153. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Ferns and Fern Allies.* By John T. Mickel. Pp.229. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95.

THE five volumes of the popular, economically priced, *How to Know* series reviewed here have certain features of format and organisation in common upon which I wish to comment beforehand. They are intended to serve the amateur naturalist and the serious student, as well as the professional scientist. In contrast to the pocketbook format of earlier editions (preferred by me for the books intended as field guides), they are of notebook size (19 × 23 cm). The paperback versions have a spiral-coil back, and a rather frangible cardboard cover not very suitable for the rigours of fieldwork. Following the instructive introductory chapters, the body of each book is comprised of abundantly illustrated keys. The final pages are assigned to a combined index and, usually pictured, glossary. In my opinion, ease of finding would be enhanced if these last two items were separated.

As the two earlier versions of *How to Know the Grasses* were authored by the same renowned agrostologist (R.W. Pohl), the reader would expect an expertly written

and polished product in this third edition. He will not be disappointed. The booklet includes keys, illustrations and distribution maps for 324 of the most common grasses of the US. Most of the meticulous drawings were done by the author himself. Following the system of classification published by Stebbins and Crampton in 1961, six subfamilies are recognised. Chapters of the introduction are devoted to definitions of structural terms. A key is provided distinguishing grasses from sedges and rushes. The student is instructed on how to collect and prepare scientific specimens. A list of 29 references suggests supplementary literature. Preceding the artificial keys to genera and species is a chapter on recognising the grass tribes and a preliminary key to major groups. Although clearly written for the beginner, the book will also appeal to the seasoned naturalist and the professional plant systematist. It is an excellent field and laboratory guide and a must for any student of grasses.

Billed as the second, this is actually the

third edition of this handy guide to the North American mosses and liverworts, *How to Know the Mosses and Liverworts* (by H.S. Conard and P.L. Redfearn Jr). While the amateur will be overwhelmed with the variety of forms and hidden structural beauty of these unobtrusive flowerless plants, the professional botanist will be impressed with the careful detail shown in the illustrations, as well as the endeavour for scientific accuracy apparent in the organisation of the contents. Introductory chapters deal with life cycles, diagnostic characteristics, habitats, preparation of specimens, and classification of categories above the level of family. Approximately 1,700 species of bryophytes occur in North America, and all the common ones can be identified using this booklet. Since the previous edition, 112 species and 42 genera have been added. The strictly dichotomous keys are broken up so that the user first arrives at the generic name and then finds the species under each genus. A brief descriptive paragraph is provided for each species. As recent taxonomic revisions have resulted in a considerable number of name changes, a list of synonyms is included. A bibliography of 35 entries inspires background and supplementary reading. The book is a treasure as a field guide, and it is definitely orientated to be a text in an introductory bryology course.

How to Know the Lichens by M.E. Hale is an attractive field and laboratory guide to the identification of lichens. It is a revised and an enlarged version of the first edition prepared, after an interval of 10 years, by the same author. There is little difference between the two editions in general outline and organisation. The geographical region considered is the North American continent but, as many lichens have a circumboreal distribution, the keys are largely applicable to the lichen flora of the entire Northern Hemisphere. Seventy new entries have been added since the first edition, bringing the total to 427. Only foliose, fruticose and squamulose lichens are treated, the crustose forms having been omitted "because they are too numerous and too little known". In the introduction, the student is informed about the symbiotic nature of lichens, about their economic uses and their sensitivity to air pollution. Several well illustrated pages are devoted to chemical, colour and crystal tests which are so essential for the identification of lichens. The student is further instructed in the use of technical terminology and in methods for storage and culture of specimens. A list of 37 references gives sources for additional information. All keys are dichotomous and conveniently broken up. Keys for the main sections are preceded by alphabetic lists of genera with a citation of their salient features. In addition to descriptions and illustrations for the major species, inset maps indicate their ranges of distribution. The keys are followed by a list

of synonyms and a classification of genera and species. The booklet constitutes an authoritative text well suited for any course on lichen systematics.

How to Know the Seed Plants by Arthur Cronquist, is a successor to the section on seed plants in H.E. Jaques' *Plant Families: How to Know Them* (1949). Approximately 375 families of seed plants are recognised and those represented by plants growing spontaneously or frequently cultivated in the US are treated in the dichotomous keys. Each family is identified by both vernacular and scientific names. In addition to a description, each entry gives information on common genera and on geographical distribution. One or more of the characteristic species are illustrated. Whereas I consider the booklet a worthwhile addition to my botanical library, I doubt whether the uninitiated amateur, who is so obviously addressed in the rather perfunctory introduction, will find it all that attractive. Amateurs have generic concepts; they may think, for example, in terms of cottonwood, lilac, clover, carnation, and so on, but would not ordinarily view these plants as members of Salicaceae, Oleaceae, Fabaceae, and so on. The book is not, nor intended to be, a substitute for an amateur's field guide. In my opinion, the introduction is aimed at too low a level of scholarship, and falls short of giving adequate background and technical instruction to potential readers who really would appreciate a text with pictured keys to the families of seed plants, namely college students and their instructors, as well as reasonably well informed naturalists. The brief chapter on principles of classification and nomenclature does not do justice to either the excellent keys or the phylogenetic outline of classification. The omission of a bibliography on background and supplementary literature is hardly forgivable and definitely impairs suitability

of the book for college-level courses on plant identification and systematics.

It is an inspiration to leaf through a scholarly prepared text like *How to Know the Ferns and Fern Allies*, by John T. Mickel. The volume succeeds in fulfilling the need for a modern field guide to all the North American petridophytes. To accommodate the amateur, technical terminology has been minimised, but without sacrificing clarity and accuracy. As emphasis rests on identification, family names are avoided and, within the major groups, the plants are keyed directly to the generic names. Species are treated in secondary keys under the alphabetically arranged genera. Identification is aided by diagnostic drawings, as well as inset maps indicating geographical distribution. Relationships among North American fern genera are presented diagrammatically at the beginning of the text. Several chapters preceding the keys furnish a comprehensive introduction to definitions of structural characteristics, life cycles, hybridisation and cytological phenomena, techniques for spore culture, as well as instruction on how to establish a fern garden and how to prepare herbarium specimens. Valuable bibliographical references are given not only at the end of some chapters, but also in the form of a list of 54 state and regional identification manuals. Preceding the index and glossary is a checklist of North American ferns and fern allies. The book is highly recommendable as a text in college courses at any level; it will also be regarded as a valuable reference work by naturalists and professional scientists dealing with the vascular cryptogams. □

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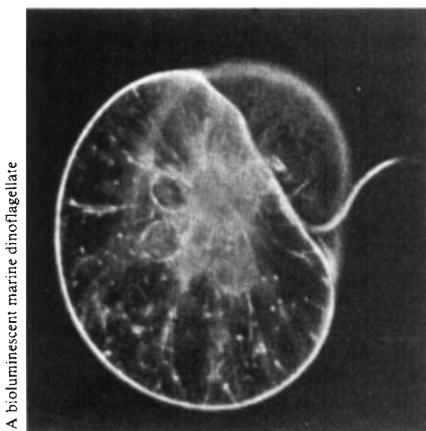
Introduction to phycology

M. G. Kelly

Introductory Phycology. By F. R. Trainor. Pp.525. (Wiley: New York and Chichester, UK, 1978.) \$21.50. *Biology of Seaweeds/Levels of Organization.* By A. R. O. Chapman. Pp.134. (University Park Press: Baltimore, 1979.) \$10.75. *Introduction to the Algae: Structure and Function.* By Harold Bold and Michael Wynne. Pp.706. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$36.40.

ALGAE are responsible for most of the energy input to marine and freshwater communities. They play a major role in controlling water chemistry, especially of oxygen, pH and nutrients. The types of algae in a water body often determine the species composition of the higher trophic levels. Algal productivity controls the locations of the world's major oceanic fisheries. In lakes, too few algae mean a poor fishery sport while too many mean extreme water quality problems. It can be easily argued that algae are fully as important as the land plants.

As Trainor points out in *Introductory Phycology*, these factors have led to an increased interest in algae, not only on the part of biologists, but also by water quality



A bioluminescent marine dinoflagellate

From *Biology* by Helena Curtis, reviewed on page 106

engineers, geochemists and others. This increased interest has produced a need for modern texts on phycology: these three respond to that need.

The shortest of these books, *Biology of Seaweeds/Levels of Organization* by Chapman, is the most specialised. Although it deals only with the macroscopic algae of coastal areas, it provides much basic information for a general algology course. The other two texts are comprehensive, in that they cover all of the phylogenetic groups. They are also 'classical' in approach, proceeding group by group, emphasising the taxonomy, morphology, reproduction, and to a much lesser degree physiology, biochemistry, ecology and distribution. Chapman, on the other hand, proceeds on the basis of levels of organisation, progressing from cellular ultrastructure through the ecology of communities. I find Chapman's approach much more appealing. It provides a holistic understanding of 'how algae work', that is, how they are adapted to exist and function within an environment. The book emphasises ideas and leads a student's train of thought within an organised framework. The approach of the other two books leads to rote memorisation and a fragmentation of ideas which students (and thoughtful professors) may find less than appealing. On the other hand, the book by Trainor and *Introduction to the Algae: Structure and Function* by Bold and Wynne are organised in the way many algology courses are taught, and this alone will probably increase their acceptance.

All three texts differ in the way algae are divided into divisions and classes. Trainor uses five divisions, and Bold and Wynne use nine. Chapman discusses three (those containing marine macroalgae), and simply avoids mentioning the controversies of classification by usually referring simply to red, brown and green algae. His approach seems most sensible. Why teach a scheme of classification and set of terminology that authorities disagree upon and that will probably be completely revised in a few years? Bold and Wynne and also Trainor recognise this problem. The former state "... 'nature mocks human categories' and this certainly seems

to be the case when one compares the more or less current systems of classification ...". Trainor quotes Dodge as stating that classes "... are the only stable feature which has persisted through the numerous classification schemes of the past few years". Perhaps both books should have followed Dodge's suggestion — don't confuse students by using conflicting classification schemes at the division level.

The two classically organised texts, Trainor, and Bold and Wynne, are very different in the amount of detail presented. Trainor is sparsely referenced and reads like transcripts from a set of very lucid lectures. Wynne and Bold, on the other hand, include 87 pages of references and the text, in places, reads like a monograph. Both provide generic examples, but Trainor's are often very sketchy while Bold and Wynne's are usually too detailed for teaching use. The difference is illustrated by the discussion of the genus *Hormotilopsis*, a somewhat obscure green soil alga first described by both Trainor and Bold. Both texts refer to it. Trainor uses it as an example of dispersion of unicells derived from planospores, but says nothing else about it so that it remains an unassociated Latin name in a student's mind. Bold and Wynne, on the other hand, feel they must tell the student that it is distinguished from *Hormotila* by having quadriflagellate rather than biflagellate zoospores, provide several illustrations, and tell when and by whom it was first isolated. I think most algologists would agree that a student would not be deprived if he never heard of either genus.

Chapman's book, published by a fairly small firm, contains few, if any errors in composition and editing. The other two, published by major houses, contain major errors. For example, in Bold and Wynne a block of text is missing on page 5. In Trainor figures 17-6 and 17-7 are identical. The caption for one states it shows algae washed ashore after a storm, while the other is supposed to be the intertidal of the Arctic; reproduction of the illustrations was so poor I was unable to tell which, if either, was shown.

A major reason for the increase of interest in algae is recognition of the importance of their ecology. Bold and Wynne deliberately avoid this topic, Trainor includes material, but much of it is solely descriptive and out of date, and nearly all would be covered in an introductory ecology course. Chapman, within his limited scope, provides an outstanding and stimulating discussion.

In summary, I will recommend Trainor to a student who wants to read about algae without taking a formal course. Bold and Wynne will become a well worn member of my reference shelf and will be owned by all of my graduate students. Finally, I look forward to someone writing a text with the organisation, style and balance of coverage that Chapman has but which covers all of the important groups. □

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Entomologically speaking

R.W. Thorp

How to Know the Insects. Third edition. By Roger Bland and H.E. Jaques. Pp.409. (Brown: Dubuque, Iowa, 1978.) \$7.95. *How to Know the True Bugs*. By J.A. Slater and R.M. Baranowski. Pp.256. (Brown: Dubuque, Iowa, 1978.) \$6.95. *How to Know the Spiders*. Third edition. By B.J. Kaston. Pp.272. (Brown: Dubuque, Iowa, 1978.) \$6.95. *How to Know the Mites and Ticks*. By Burruss McDaniel. Pp.335. (Brown: Dubuque, Iowa, 1979.) \$7.95.

Two new volumes and two revised third editions dealing with insects and arachnids have been published in the *How to Know* series since 1978. All four have a plate size 2×4.5 cm larger than earlier editions with an easier to read double column format. One problem with the volumes on true bugs, spiders, and especially mites and

ticks is that descriptions of genera, species and keys to species are inserted among the couplets of the keys to genera, occasionally separating halves of these couplets by up to two pages. Since many of the taxa treated in these volumes are cosmopolitan, they may prove useful as identification manuals in other countries.

In most particulars, the edition of *How to Know the Insects* by R.G. Bland and H.E. Jaques is a vast improvement over the second edition: more than half again as many families are keyed; families of scale insects (Coccoidea) are not keyed, but are discussed and diagnosed; family discussions include more complete descriptive and biological data; many subfamilies of large families of Orthoptera (Acrididae, Tetigoniidae, Gryllidae), Coleoptera (Scarabaeidae, Chrysomelidae, Cerambycidae, Curculionidae), Lepidoptera (Saturniidae, Arctiidae, Pyralidae) and Hymenoptera (Formicidae, Vespidae, Sphecidae) are diagnosed and discussed, although keys are not provided; there are many more illustrations and their larger size, improved quality and detail make them much more useful; the classifications are more current and orders and

families are organised in a more closely phylogenetic sequence; although illustrations are not included in the terminal Index and Glossary, references to appropriate text figures are found with each term defined. Missing are the useful systematic lists of families of North American insects for each order. Also gone are the folksy humour and enthusiasm of the initiator of the series, Professor Jaques, but these are replaced by more precise and erudite discussions of the diversity and interest of insects.

Introductory chapters on how to collect, preserve, mount, curate, observe, rear and where to look for insects are concise and well done. The chapter on structure and development is brief, but does seem to cover the bare essentials of structure necessary to use the keys. The keys are well constructed, with a minimum of technical jargon allowing easy use even by a novice. A key to orders of adult insects is followed by treatments of each order.

Each order is introduced with a brief descriptive diagnosis, habits, life histories, habitats occupied, special collecting and curating techniques, and estimates of the numbers of species and families in North America and species worldwide. This is followed by a key to the common families of the order. The family key is followed by brief structural diagnoses of each family, their principal habitats and often by brief descriptions of the most common species. A list of general references usually concludes each ordinal treatment.

Few errors of any import were noted. The reference to one of the best new textbooks on entomology by H.V. Daly, J.T. Doyen and P.R. Ehrlich, *An Introduction to Insect Biology and Diversity* (McGraw-Hill: New York, 1978), is curiously included within the reference to the book by M.D. Atkins. Missing from the same list of general references in insect biology is another important multi-authored book edited by D.F. Waterhouse (*The Insects of Australia*. Sponsored by CSIRO, Canberra. Melbourne University Press, 1970). Although the key to Mecoptera families correctly states that Bittacidae have only a single large claw on each tarsus, figure 223A clearly shows two tarsal claws on each leg of *Bittacus apicalis* Hagen.

The quality of the illustrations in this volume is not on a par with those in the better known field guides. However, it does provide keys for identification which the field guides usually do not and the keys are simpler and easier to use than those of the more expensive general texts. Although this volume lacks lists of family group and higher classificatory taxa for each order, its coverage of over 60% of the families of America north of Mexico, its clear, concise, accurate keys and descriptions, and its relatively low price make it an attractive alternative to the texts for field and laboratory courses devoted to identification and classification of adult insects.

How to Know the True Bugs by J.A. Slater and R.M. Baranowski is an excellent addition to the series, and includes keys to the families of Hemiptera-Heteroptera of North America, to 475 genera, and to 65 species and 2 subspecies in 10 of these genera. In addition to the usual introductory material on life history and morphology, and collecting, preserving and labelling methods, a special historical section introducing early European and American hemipterists is included. Following the key to families based on adult Hemiptera is a separate key to families based on nymphs. The majority of the book consists of family treatments introduced by brief descriptions and biological information, most followed by a key to genera with brief discussions of each genus often followed by descriptions of common species. Most of the family treatments end with references to pertinent literature. At the end of the book is a list of common names of true bugs as approved by the Entomological Society of America, followed by the standard index and glossary.

Few errors of any import were found. In the key to genera of Coreidae the genus *Thasus* was apparently omitted from the first half of couplet 2. The illustrations are of excellent quality. The keys are clear, concise and easy to use. This volume will be useful to all who have occasion to identify the genera true bugs. I know of no other single reference which serves this need for all of North America.

The larger format of the new edition of *How to Know the Spiders* by B.J. Kaston has increased the size of the illustrations and improved their clarity, quality and usefulness, especially those showing webs and snares. More than 40 new figures have been added, and the cartoons of the second edition have been deleted. A new chapter presenting a pictured key to the orders of Arachnida increases the utility of this volume, and places spiders in the context of their closest relatives. One of the more useful features of the second edition which has been carried forward into the current edition is the list of families of spiders arranged in their higher classification groupings and with footnotes denoting families which have representatives in America north of Mexico. The bulk of the book is devoted to descriptions of the families and keys to the more common genera. The additional genera and species treated in this volume bring the coverage to nearly double that of the original volume. Since nine families are uncommonly collected, they are only briefly discussed at the point where they come out in the key to families and therefore do not appear in the list of families in the table of contents.

The volume could be tightened up a bit by eliminating repetition of several figures, some of which are reprinted on four separate pages (for example, *Atypus niger* ventral view is used for Figs 26, 49, 63, 160), where a single figure with subsequent

references to it would suffice. Nonetheless, this volume remains the best resource for identification of North American spiders.

McDaniel should be complimented on his attempt to produce in *How to Know the Mites and Ticks* an identification manual for such a taxonomically difficult group. Especially creditable is the key to families and genera of the Oribatei. Most of the figures are well done and useful. However, figures 1-3 in the section on morphology could stand enlargement and the captions are missing for some (for example, 12, 13, 18, 25, 99-102, 113) or separated by a column or even a page from the figure they belong to. Hypopi of astigmatid families (for example, Acaridae, Anonettidae and Glycyphagidae) are figured and some are diagnosed.

This volume would benefit from the inclusion of a list giving the higher classification (order to families) rather than referring the reader to the excellent manual by G.W. Krantz (*A Manual of Acarology*, 2nd edition, OSU Book Stores: Corvallis, Oregon, 1978). This volume suffers from uneven treatment of taxa with considerable space devoted to such families as Pyemotidae, Chyletidae and Listrophoridae while economically and medically important large families such as Eriophyidae, Tetranychidae and Trombiculidae have only one species treated. The so-called keys to genera of many families are not dichotomous keys with contrasting couplets, but rather a sequential series of generic diagnoses (for example, Spinturnicidae, Tarsonemidae, Pyemotidae, Chyletidae, Tenuiplapidae, Stigmaeidae, Tydeidae, Saprogllyphidae, Psoroptidae, Glycyphagidae, Anonettidae, Pyroglyphidae, Listrophoridae, Sarcopidae, Epidermoptidae, Chirodiscidae, Myocoptidae). The key to species of *Ornithodoros* should be set in a smaller typeface with different couple designations so as to distinguish it from the surrounding key to genera of Metastigmata (compare keys to species of *Ornithonyssus* and *Psoroptes*).

Although this volume is unlikely to replace or even supplement the more expensive manual by Krantz in laboratory courses in acarology, it will prove a valuable tool for less specialised courses dealing with terrestrial arthropods, and for state and federal workers and other researchers who have occasion to make preliminary sorts of mites to be sent to specialists for determination. □

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A tiger beetle

Animal behaviour

J.P. Hailman

The Ecology and Evolution of Animal Behavior. Second edition. By Robert A. Wallace. Pp.284. (Goodyear: Santa Monica, California, 1979.) Price please. *Animal Behavior: An Evolutionary Approach.* Second edition. By John Alcock. Pp.532. (Sinauer Associates: Sunderland, Massachusetts, 1979.) \$16.00 cloth. *Evolution of Behaviour.* By Jerram L. Brown. Pp.760. (W.W. Norton: New York, 1975.) \$17.95. *Insect Behaviour.* By Robert W. Matthews and Janice R. Matthews. Pp.507. (John Wiley: New York and Chichester, UK, 1978.) \$32.40. *Comparative Animal Behaviour.* By Donald A. Dewsbury. Pp.452. (McGraw-Hill: New York and London, 1978.) \$17.95.

THE closing year of the decade added at least three textbooks on the increasingly popular study of animal behaviour. Robert A. Wallace's *Ecology and Evolution of Animal Behavior* appears in a second edition, with 16 chapters embracing such topics as "classical instinct theory", drive, learning, communication, sex, aggression and social behaviour including altruism and warfare. The frontispiece is clearly a discus (*Symphysodon*), and on page 118 where the figure is repeated we learn that it illustrates "false eyespots near their tail."

The figure might be symptomatic of a second major problem of this book: the common discus species known to aquarium buffs (*S. aequifasciata* and *S. discus*) have no such eyespots, I cannot find such a marking in illustrations of *Symphysodon* species, and an ichthyologically knowledgeable colleague knows of no such species. It would have been useful for the book to identify all species illustrated. Surely the "peregrine falcon" in figure 2.2 is peculiar for the species, and in any case is landing rather than hunting as asserted. Sea horses (figure 2.5) suck up prey somewhat like a vacuum cleaner does, rather than snapping them as asserted. Although some instructors may like the chatty, anecdotal nature of the text, I find it slightly condescending and certainly no paragon for undergraduate writing.

John Alcock's widely used *Animal Behavior: An Evolutionary Approach* appears in a second edition that shows some, but not marked, improvement over the first. This is not a general text on behaviour, but rather is aimed at ecological and evolutionary aspects. There is some material on immediate control of behaviour and its physiological mechanisms, but the author deals only briefly with learning and ontogenetic

development. Indeed, the brief treatment of ontogeny is totally puzzling, as evidenced by such sentences as: "Please note that I am NOT arguing that innate behavior patterns are genetic or more genetic than learned behaviors." When Alcock is in his evolutionary element, however, the text is extremely good, and I believe that a perceptive instructor could utilise it in conjunction with other material.

Because of the similarity with Alcock's book, Jerram L. Brown's *Evolution of Behavior* deserves at least passing mention. The focus is the same, but there is more material on both physiological mechanisms and ontogenetic development of behaviour, making the book more comprehensive, if a few years older. Like Alcock and most other evolutionary behaviourists, Brown finds environmental influences on the development of behaviour slightly annoying, but at least he faces the annoyance reasonably honestly rather than trying to explain it away with semantics.

For entomological ethologists the new *Insect Behavior* by Robert W. and Janice R. Matthews is the first real text of its kind. Although I claim no expertise in insect behaviour, I found the organisation of this book well suited to its subject matter, and those subjects that I know something about to be treated well. Finally, one need mention Donald A. Dewsbury's *Comparative Animal Behavior*, a book (by

a psychologist) with too much elementary zoological material but with a marvellous balance of treatment among the four classes of behavioural determinants — control, ontogeny, perpetuation and phylogeny of behaviour. The text is not, however, orientated substantially toward natural history and field studies, so has not earned plaudits from my students to match my own.

It is difficult to escape the conclusion that a really outstanding text on animal behaviour has yet to be written for a modern audience. Many teachers still rely on one of the three originals, two in revised editions: *Mechanisms of Animal Behavior* by Peter Marler and William J. Hamilton III (Wiley: New York and Chichester, UK, 1966), *An Introduction to Animal Behavior: Ethology's First Century* by Peter H. Klopfer (Prentice-Hall: Englewood Cliffs, New Jersey, 1974) or *Animal Behaviour: A Synthesis of Ethology and Comparative Psychology* by Robert A. Hinde (McGraw-Hill: New York, 1970). Each has its own strengths and weaknesses and all have been well reviewed over the years. A cynic might prefer to follow the dictum of Louis Agassiz: "Learn from nature, not books."

□

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Introductory chemistry for the health sciences

A.D. Cooper

Chemical Principles for Life. By Lois Forney. Pp.574. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$14.95. *Elements of General and Biological Chemistry.* Fifth edition. By John Holum. Pp.571. (Wiley: New York, 1979.) \$16.95. *Chemical Principles for the Life Sciences.* Second edition. By Ralph Fessenden and Joan Fessenden. Pp.526. (Allyn and Bacon: Boston, 1979.) \$18.95.

ALL three texts are well written. Forney writes at a higher level than the other authors. Particularly difficult concepts covered by Forney are: thermodynamics, Henderson-Hasselbach equation, coupled reactions, standard reduction potentials and titration curves. Difficult concepts are often covered relatively early in the text.

Holum writes at a lower level than the others. Fessenden and Fessenden write at an intermediate level that can be understood by most students. The authors are patient and approach each topic in a systematic way. Difficult concepts covered by the Fessendens are equilibrium constants and coupled reactions. In general, Forney and the Fessendens define terms more clearly than does Holum.

Of the three texts, Holum's has the most special features. Each chapter has a summary, selected readings and ample questions. Worked out examples are included and answers to in-chapter exercises are at the back of the book. Appendices include mathematical manipulations, inorganic and organic nomenclature, and data on nutrition. At the end of each chapter the Forney text has a programmed review that includes fill-in-the-blank questions with answers in the left-hand margin. Some of the chapters have additional questions and mathematical problems. Worked out examples are included. No appendices are given. The Fessenden text has a set of objectives at the start of each chapter and study questions at the end. Numerous well

worked out examples are included. The appendix on mathematical manipulations is thorough. Answers to odd-numbered study questions are at the back of the book.

The Holum text is well illustrated, with many pictures and coloured plates. However, the illustrations in the Forney and Fessenden texts are more than adequate. The figures of Forney's text are in black with shades of grey. Those of the Fessenden and Holum texts are in shades of blue and grey with section headings in blue. The Holum text contains 22 chapters whereas the Forney and Fessenden texts contain 31 and 30 chapters, respectively. The order of chapters and topics are similar with few exceptions. Nuclear chemistry and radioactivity are treated in Chapter 5 by Forney, in Chapter 10 by the Fessendens and in Chapter 22 by Holum. The treatment of electrolytes, body fluids and acid-base balance is presented in the general chemistry unit by Forney whereas these topics are treated in the biochemistry unit by the Fessendens and Holum. The Fessendens have separate chapters on oxidation-reduction reactions, introducing organic chemistry, introducing biochemistry, molecules in three dimensions (optical isomerism), and a chapter entitled Minerals, Vitamins and Drugs. In contrast, Holum and Forney have only the chapter on introducing organic chemistry.

All three texts give a good treatment of the principles of general chemistry, with Fessenden and Fessenden giving the most careful and straightforward presentation. An integral part of many introductory chemistry courses is mathematical problem solving. The Forney and Holum texts give a modest number of mathematical problems. The Fessenden text gives a much more quantitative treatment than the others. Of the three, the Holum text gives the most abbreviated treatment of organic chemistry. All three cover the various principles of biochemistry in a traditional manner.

Throughout these texts relevant examples and applications from the health science fields are described. Forney gives the most applications, with Holum a close second. The Fessendens' use of relevant examples is more judicious and certainly more than adequate.

Outstanding features of these books include the following. Holum's use of up-to-date relevant examples such as clinical charts and a section on radioimmunoassays. The Fessendens' chapter on molecules in three dimensions is excellent. Forney's discussion of several mechanisms of organic chemistry is commendable because it gives students a rationale for predicting products of reactions.

Weaknesses of these texts include the following. Holum gives very few problems concerning the gas laws and he omits a discussion of competitive versus non-competitive inhibition. His treatment of stereochemistry is very brief. The

Fessendens place the chapter on aromatic hydrocarbons near the end of the unit on organic chemistry. Forney does not include any worked out examples on organic nomenclature and writing structural formulae. Her discussion of stereochemistry in the introductory chapter is much too early.

In summary, the Fessenden text is clear and straightforward. It emphasises chemical principles, contains numerous worked out problems and has a modest number of relevant examples from the health sciences. This text is suitable for a course emphasising a traditional chemistry approach. Of the three, the Holum text has the best blend of worked out problems, relevant examples and chemical principles. It would be suitable for a course requiring a

modest degree of mathematical problem solving, a short treatment of organic chemistry and a lot of relevancy. Forney's text is written at a high level and is demanding of the student(s). It would be suitable for a course that emphasises the chemical principles involved in physiological processes and requires only a modest amount of mathematical problem solving. For our two-semester course designed primarily for students in our Nursing Education Program I would prefer the text by Fessenden and Fessenden. □

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Physical chemistry for biologists

Verne N. Schumaker
E. Reisler

Physical Chemistry and its Biological Applications. By Wallace S. Brey. Pp.589. (Academic: New York, San Francisco and London, 1978.) \$15.95.

SOME, but no detailed knowledge of calculus is required for the study of this text, designed to present the basic principles of physical chemistry with the biologist in mind. The selection of topics is good and the book is easily read. Although the level is elementary, biology majors may find it challenging and it should be considered by instructors who plan to teach at this level.

The first two chapters introduce the kinetic theory of gases, intermolecular forces, Raoult's and Henry's laws, and the colligative properties of solutions. This material expands upon topics usually introduced in first year chemistry courses at about the same level of sophistication. The basic concepts of thermodynamics are presented in the next two chapters. In our opinion these chapters will require much discussion and supplementation by the instructor to impart more than a casual acquaintance with the first and second laws.

A good presentation of the Debye-Huckel theory is given in the chapter on solutions of electrolytes. The elementary algebra of ionisation and titration of weak electrolytes comes next, followed by a

chapter on oxidation and reduction. Biological applications are described mostly in qualitative terms. The electromagnetic and quantum nature of radiation is used to introduce a simple exposition of the photoelectric effect, the Bohr atom, particle spin, and the structure of polyatomic atoms. The chapter on quantum mechanics ends with a presentation of the Schrödinger equation.

The discussion of bonding and molecular spectroscopy is a descriptive overview of these important areas of physical biochemistry. The explanation of covalent bonds is based on the concept of MO, but the LCAO method, although employed in the text, is never explicitly described. Whenever specific examples are used to clarify the introduced concepts (hybridisation of orbitals, electron delocalisation, vibrational spectroscopy) the presentation is clear and helpful. Fluorescence, for all its potential uses in biochemistry, is hardly mentioned. Circular dichroism, though more popular nowadays than ORD, receives less attention than the latter. The qualitative explanation of these techniques is clear, though it lacks somewhat the continuity of presentation.

The chapters on Kinetics of Chemical Reactions and on the Adsorption and Surface Effects are well written. Though brief, these sections focus the attention on areas of great significance. Transport methods and light scattering are described in the chapter on macromolecules. Specific examples or additional illustrations would have helped in giving the students a better feeling for how the actual experiment is conducted, and the data collected and calculated. □

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Introductory biology texts

Edward C. Cox & Robert M. May

Biology. By Karen Arms and Pamela Camp. Pp.970. (Holt, Rinehart and Winston, 1979.) \$17.95. *Life on Earth*. Second edition. By Edward Wilson *et al.* Pp.846. (Sinauer: Sunderland, Massachusetts, 1978.) \$16.95. *Study Guide for Life on Earth*. Second edition. By P.S. Associates and Carl Pike. Pp.281. (Sinauer: Sunderland, Massachusetts, 1978.) \$5.95. *Test File for Life on Earth*. Second edition. By P.S. Associates and Carl Pike. Pp.97. (Sinauer: Sunderland, Massachusetts, 1978.) Free. *Biology*. Third edition. By Helena Curtis. Pp.1043. (Worth: New York, 1979.) \$19.95. *Biology*. Second edition. By James Case. Pp.673. (Macmillan: New York, 1979.) \$17.95. *Biology, The World of Life*. Second edition. By Robert Wallace. Pp.584. (Goodyear: Santa Monica, California, 1979.) \$16.95. *Biology, The World of Life: A Learning/ Study Guide*. Second edition. By Gerald Sanders. Pp.200. (Goodyear: Santa Monica, California, 1978.) \$7.95. *Concepts in Biology*. Second edition. By Eldon Enger, Andrew Gibson, J. Richard Kormelink, Frederick Ross and Rodney Smith. Pp.512. (Brown: Dubuque, Iowa, 1979.) \$13.95. *The World of Biology*. By P. William Davis and Eldra Pearl Solomon. Pp.756. (McGraw-Hill: New York, 1979.) \$16.50. *Biology Lab Manual*. Sixth edition. By A.M. Winchester. Pp.331. (Brown: Dubuque, Iowa, 1979.) \$10.95.

In the US, there are around 3,000 colleges and universities, ranging from local, 2-year junior colleges to the 100 or so major research universities. The array is, moreover, a multi-dimensional one; entry standards vary widely, and some of the small colleges produce very little research yet do some of the best teaching, while some of the great research universities tend to process, rather than teach, all but a few of their students. This enormous and healthy diversity means that introductory biology courses are taught at many different levels, and college texts are competing in a multifaceted niche space. This is reflected in the character of the introductory biology texts reviewed here.

With differences in tactical detail, all seven books share a common strategy for organising the material. This "little-to-big" strategy begins with the rudiments of chemistry and molecular biology, and proceeds to cellular, organismal and population biology, ending with discussion of human ecology. We now comment briefly on the individual books (taking them in what we judge to be descending order of sophistication), and then air some general opinions.

The book by Arms and Camp is the longest (970 pages of double-column small print) and most consistently scholarly of the seven. The authors explain their aim is

to write a comprehensive text without the usual heavy emphasis on molecular biology, giving a fair share of attention to plants, adaptation and ecology; plants, particularly, tend to get short shrift in introductory texts, as instanced by the other six books here. Arms and Camp's *Biology* has six main sections, beginning with cells, and going on to information coding and transfer, taxonomy and discussion of the major phyla, animal biology (mainly physiology, with two excellent chapters on neurobiology), plant biology, and ecology and evolution. The information is up to date (for example, Chapter 9 treats recent work on recombinant DNA, and ends with two incisive essays on protein sequencing and evolutionary trees), and the many illustrations are cogent. The section on ecology and evolution is comprehensive enough to serve as a text for a basic course specialising in that subject, a claim that could not be made for any of the other books.

Generally, Arms and Camp's *Biology* has an air of authority (surpassed only by the high points in *Life on Earth*). The book is also a well designed learning tool, each chapter beginning with a list of objectives and questions, and ending with a self-quiz, a thoughtful set of "questions for discussion", and references for further reading. One fault is a tendency to excessive codification of organisational structure of the book; this reaches obsessive proportions in the 8-page, double-column table of contents. While one of us (R.M.M.) is greatly addicted to numbering things, it must be admitted that: (AC-1) although pedagogically sound, the practice (AC-2) irritates most students, and (AC-3) is here done in an aesthetically displeasing way. Apart from this minor flaw, and the tendency to excessive compendiousness, the book is an excellent and well balanced text for introductory biology at a good college, covering the field at the right level and in a serious and interesting way.

Life on Earth by Wilson *et al.* is the book we have been using in our own course. The good parts are very good indeed, but (as is hard to avoid with seven authors) the book is patchy both in the topics covered and in the level of presentation. In general, the chapters on cell chemistry, the structure of macromolecules, energetics, population biology and ecology are done well. But parts of the chapters on molecular genetics, classical genetics, and cell chemistry and development are superficial, use metaphors that distract rather than enlighten, or are excessively compressed (sometimes to the point of unintelligibility). The quest for "relevance" is not always fortunate; the two final chapters on alternative futures, "Toward A Stable Ecosystem" and "Defeat by Default", are troughs of insubstantial rhetoric. The book by Curtis has the edge in careful planning, consistent presentation, and crisply appropriate examples from the literature. The strengths of *Life on Earth* are the peaks where people close to the frontier give glimpses that a well prepared student will find new and exciting: the evolution of polypeptide chains; the origins of life on Earth; the molecular details of enzyme action; island biogeography.

Two booklets accompany *Life on Earth*: a *Study Guide* and *Test File*. The *Test File* is simply a compendium of over 1,000 multiple-choice test questions. It is easy to find horrors, but then it almost always is. Chapter by chapter, the *Study Guide* summarises key ideas and terms, gives a quiz for "organizing and testing your knowledge" (which is done well), and gives a sample "self-test". The emphasis is on regurgitating information (as Simon and Garfunkel sing, "All my words come back to me, in shades of mediocrity"), which is not easily avoided in introductory biology texts. Diligent use of these booklets would almost guarantee a student an A in a multiple-choice or short answer examination.

The third edition of *Biology* by Helena Curtis has moved from strength to strength through two substantial revisions. The book has the standard progression from the biology of cells, through the biology of organisms, to the biology of populations. Uniformly, the coverage is thorough in simple and lucid prose, making few assumptions and basing the discussion on the previous chapters. The approach tends to be intuitive, rather than analytical. The book is richly illustrated by spectacular micrographs, colour photographs and excellent graphics. The diagrammatic representation of the Krebs' cycle, for example, uses three colours to lay bare the essentials of the cycle with brilliant clarity. At no point, however, does the book strive for real depth or authority. Beautifully produced and even in tone, it is an extremely good introductory biology text for students who have no extensive science background; but it may lack the spark desirable in a college text for students who



A spider monkey

From *Biology* by Helena Curtis

have a first-rate high school preparation in the natural sciences.

Case's *Biology* is a well written text at the level appropriate for a student with relatively little science background. It begins with chemistry and the beginnings of life, and moves to genetic biology and multicellular life and (briefly) evolution. The second half of the book is devoted to human biology, mainly physiology, with brief excursions into general aspects of population biology and ecology as they bear on human populations and their environmental problems. Various topics are fleshed out nicely by quotations from the original researchers. Among the seven texts, Case deserves praise for making the most substantial attempt to tackle mathematical problems in biology, particularly in quantitative aspects of population growth. The book is well produced (though nothing rivals Curtis). It is broadly at the level of Curtis, differing in giving more attention to human biology and human problems, and less attention to other organisms above the cellular level.

In *Biology, The World of Life* by Wallace, the first 8 pages set much of the tone, juxtaposing scenes from the natural and the man-made world: row houses in San Francisco versus the hills untouched; the northwest face of half-dome in Yosemite versus trash piled in a New York alley; but not, for example, the Taj Mahal versus a naturally oily bog. The basic presentation is thorough, rigorous and well organised in the canonical little-to-big format, but lacking a sense of the excitement of the research frontier. The population biology and ecology sections are at a low level, and are broken up by intrusive moral tales. The illustrations are good, though the graphics are not as good as in the above texts. Much more than any of the other books, Wallace's text conveys a real sense of a characterful individual who really enjoyed writing it. If we must have an author's views on sex or why have children or the sociobiology debate pressed upon us, let it be someone with the sense, sensibility and self-deprecating humour of Wallace. We found his distinctive style to be, in the main, engaging and witty, with occasional lapses into the self-consciously literary or, like, cool ("We've tossed around some heavy concepts fairly loosely here", page 441). The book is designed primarily for students not majoring in biology, which seems right, although if one really warmed to Wallace's idiosyncrasies, one could risk the text on biology majors.

Wallace's book comes with a *Study Guide*, which we think clearly superior to the one accompanying *Life on Earth*. There is some reliance on true/false questions, and on multi-choice, but an attempt is made to get the student thinking as well as mastering detail. The helpful hints (for example, on how to solve genetics problems) seem genuinely helpful.

Concepts in Biology by Enger *et al.* is apparently designed primarily for use in

junior colleges. The chapters on molecular biology and cell structure and chemistry are very simple, but accurate and competently done. Discussion, for example, of photosynthesis, glycolysis and oxidative phosphorylation is to the point and easy to follow. In the absence, however, of a discussion of underlying energetics, the processes lack context and appear arbitrary; a little like memorising Persian. The presentation of organismal biology and ecology is similarly competent, adequately illustrated, but descriptive rather than analytical. The sentences are short, and the general tone seems aimed at an audience with little preparation, not only in science, but in reading or thinking; a random collection of section headings is illustrative ("The environment calls the shots", "Nature throws a curve" (the logistic), "The holey ones — the sponges or porifera", lots of irrelevant cartoons). For the chosen level, the biological coverage is good, but students deserve better prose than this.

The World of Biology by Davis and Solomon is written at a very elementary level. It is given to discussion of the "lifestyle" of organisms, and to "relevance". It also has too many mistakes, which disqualify it as a text. For example, on page 39 it is claimed that radioactive oxygen was used to show that the oxygen in CO_2 appears in glucose, not in free oxygen. Two kinds of experiments were actually done: in one, ^{18}O -labelled water was shown to generate ^{18}O -labelled oxygen by photolysis; in the other, isotopes of carbon, both stable and radioactive, were used to show that carbon from CO_2 appears in glucose. On page 78, the discussion about conformational regulation of enzymes contains a completely misleading example, implying that proteins denature and renature as they go from inactive to active forms within the cell. On page 111, apart from the slangy writing in the first part of the paragraph, it is asserted that 90% of cancer cases are thought to result from environmental factors; this is, to put it mildly, debatable. On page 609, the Scopes trial is claimed to have increased public acceptance of evolution; in fact, its immediate effect was to see references to evolution systematically removed from the next generation of textbooks.

In the table below, we have summarised some of the more mechani-

cally pedagogical features of these books. It must, of course, be remembered that the above list is not an exhaustive list of introductory texts. In particular, the new edition of Keeton will probably be a strong contender in the top category.

Our list of books also included a *Biology Laboratory Manual* by Winchester. This manual sets out 26 different experiments, illustrating many of the chapters in most introductory texts: chemistry; cell movement; seeds; protists; structure of higher plants through animals; the reproductive system; and principles of heredity. For the most part these experiments are purely descriptive, with little emphasis on doing new things or testing unknowns, or the like. We like the chapter on the reproductive system where it is noted (page 296) "Now that we have gotten away from some of the mid-Victorian ideas about anything related to sex", "semen can be collected in a condom during sexual intercourse" for this part of the laboratory. The manual offers no advice, West-Coast-contemporary or otherwise, on who supplies the sample.

Looking over this set of books, as a whole, prompts several thoughts.

The subject matter embraced by biology has grown, and continues to grow, at a prodigious rate. There is an inevitable tendency to gargantuan texts, overwhelming the student with detail and anecdote. One way of coping with this problem is to be selective in the coverage, but as a publication strategy this carries the danger that opinionated reviewers will complain about patchiness, as we have about *Life on Earth*. Another idea, easier said than done, is to focus on a few organising themes and general questions. For example, the question of why are there as many species as there are — a few million rather than billions or thousands — has nagged the seminal thinkers from Darwin to our day. Much of the material on ecology, phylogeny and the fossil record can be organised around this theme, but ultimately our ignorance about the answer to the question makes for a fuzziness that most students find uncomfortable (which is, presumably, why none of the books develops this particular theme). As another example, why do most ecosystems have only three or four trophic levels? A limited number of studies in the 1950s suggested 10% of the energy at one level flows to the

Pedagogic characteristics of the seven texts

Authors	Objectives listed at start of chapter?	Summary at end of chapter?	Glossary of technical terms?	Questions at end of chapter?	References for further reading?
Arms & Camp	Yes	Yes	Combined with index	Yes	Yes
Case	No	No	Combined with index	No	No
Curtis	No	Yes	End of book	Yes	Sometimes
Davis & Solomon	Yes	Yes	Start of chapter	Yes	Yes
Enger <i>et al.</i>	Briefly	Yes	End of both chapter and book	Yes	A few
Wallace	No	Study guide	End of book	Study guide	Yes
Wilson <i>et al.</i>	No	Study guide	End of book	Study guide, Test file	Yes

next, which gave rise to the answer that such attenuation necessarily limits the number of trophic levels to a few. But subsequent work has shown the percentage of energy transfer varies widely among terrestrial and marine communities, and between warm and cold blooded animals, yet the number of trophic levels remains remarkably constant at around three or four (with a tendency to fewer, not more, levels in highly productive environments). Here is a fascinating question, of importance and relevance. Yet only two books (Arms and Camp, and Wilson *et al.*) raise the question as such, and both answer it with the conventional wisdom of the 1950s. All seven books assert the "10% law", and the *Test File for Life on Earth*, indeed, offers a multiple-choice question in which the "correct" answer is that 10% of the energy at one trophic level is transferred to the next, with 5% or 2% being wrong answers; this is fossilised misinformation. In short, the larger questions tend to be lost, and replaced by detail.

The mathematics in these books at most extends to simple algebra and the exponential function. The student is asked to understand the simple mathematics of the Hardy-Weinberg theorem in five of the books (Davis and Solomon mention it, Case does not), but population genetics is

not further developed. It is hard to see how things could be otherwise, given the low and systematically declining (witness the SAT scores) mathematical preparation of students entering college in the US. At the same time, description continues to give way to analytical approaches in biological research, and the gap between what is needed and what we teach in elementary courses continues to widen. There is no easy answer.

With the exception of Arms and Camp, and Curtis, all the books, to one degree or another, preach to their audience about matters to which the authors bring no special qualifications. The classroom setting encourages this self-indulgent evangelism about sex, lifestyles and man's environmental problems, to the point where it constitutes a form of occupational hazard. But there is less excuse for allowing such material to pervade an introductory biology text, much less allowing it to provide the central theme. Examples are Wallace devoting more space to foreplay (2 pages) than to food chains (1 page), Case more or less equating ecology with human ecology, David and Solomon giving as much space to examining yourself for breast cancer as to the history of ideas about evolution, or the discussion of reproduction in *Life on Earth* degenerating into

chatter about Harris' *My Life and Loves*. Relevance is the grail that justifies all this. The pity is that biology in general, and population biology and ecology in particular, offer much scope for the pursuit of important and relevant problems, in a substantial way that lies within the framework of the discipline. Our bad-tempered remarks stem from the belief that the most important goals of college education are to introduce people to sustained and dispassionate analysis, and to teach them to distinguish hard fact from casual opinion; the books do not all serve this cause well.

Finally, notice that the seven books illustrate a form of law of one price. Apart from the paper-bound Enger *et al.*, the books all inhabit the range \$16.50 to \$20.00. The absence of niche segregation by price is understandable, although remarkable in view of the disparities in size, quality of production and quantity of professional effort among the books. □

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Physics for poets

A. M. Sachs

Physics Without Math: A Descriptive Introduction. By Gilbert Shapiro. Pp.351. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *The Ideas of Physics.* Second edition. By Douglas C. Giancoli. Pp.528. (Harcourt Brace Jovanovich: New York, 1978.) \$14.95. *Physical Science: A Dynamic Approach.* By Robert T. Dixon. Pp.402. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$15.95. *Conceptual Physics . . . A New Introduction to Your Environment.* Third edition. Written and illustrated by Paul G. Hewitt. Pp.660. (Little, Brown and Company: Boston, 1977.) \$15.95. *Instructor's Manual to Accompany: Conceptual Physics . . . A New Introduction to Your Environment.* Third edition. By Paul G. Hewitt. Pp.245. (Little, Brown and Company: Boston, 1977.) Free.

THE four books will be considered here as texts for an elementary and usually terminal physical science course for non-science college students, who often refer to it as physics for poets. (Giancoli's and Hewitt's books are also intended, as

Hewitt states, "as a springboard to a greater involvement in physics".) Although this reviewer is presently teaching such a course, he has neither used these texts nor had the opportunity of observing the reaction of students who have read them. On a larger scale, it is unlikely that any text will gain widespread acceptance in this area, since each teacher seems to have his own ideas in particular as to how a physics course for liberal arts students should be designed.

These four books explicitly avoid mathematics and either under-emphasise or omit entirely quantitative problems. In *Physics Without Math: A Descriptive Introduction*, Shapiro states that "it is possible to learn about the scope and power and, yes, beauty of a scientific explanation of nature without solving equations". It is possible, perhaps, but the question could be raised as to whether the psychological gain for some students with "built-in bias against science" is worth the verbal complication for others in his statement, for example, of Kepler's third law: "The ratio (distance times distance times distance) divided by (period times period) is the same for all planets." Giancoli in *The Ideas of Physics*, does use equations in the text and gives some derivations in footnotes, and — presumably to broaden the prospective market — indicates that an "Optional Mathematical Supplement" is available.

All the books cover broad areas of physics, with a varying proliferation of topics. In common with most introductory physics texts, there is little continuity between sections. The second half of Dixon's book *Physical Science: A Dynamic Approach*, is devoted to a survey of chemistry, geology and astronomy, so the 200-page coverage of most areas of physics in the first half is probably too superficial for a course devoted entirely to physics. Giancoli's book and Hewitt's *Conceptual Physics . . . A New Introduction to your Environment*, follow the conventional order and exhaustive list of topics of traditional texts, including fluids, sound and geometrical optics, as well as introducing relativity. Shapiro does not hesitate to omit some topics entirely and to skip over the details of others, while concentrating on introducing enough physics to permit discussion of major areas such as energy and the 'frontiers of science'.

All the authors stress to some extent the historical development of science, as, for example, in the familiar treatment leading from the description of planetary motion to the formulation of newtonian mechanics. Shapiro and Giancoli are a little more consistent in maintaining a historical perspective, but most of the references after the eighteenth century are primarily to names and dates.

Shapiro is concerned with the basic

concepts of physics; a limited number of specific phenomena are described, primarily as examples of these concepts. Hewitt is more directly concerned with 'how things work' and describes, somewhat superficially, many devices such as an aneroid barometer, microelectronic circuits, a ruby laser and a fluorescent light. Giancoli takes more of a traditional approach, stressing important concepts but with a tendency toward describing a wider variety of phenomena.

The four books vary considerably in their scientific sophistication, the liveliness of their presentation and the visual appeal of their format. Shapiro's book displays a deep understanding of physics, with a number of unconventional approaches such as introducing mechanics through the concept of energy and defining force, pressure, surface tension and torque as ratios of "energy transferred" to distance, volume decrease, surface increase and angle, respectively. A serious student can gain significant insight from the book, especially if guided and reinforced by an

accompanying course; less intensive reading of the book, however, is likely to lead to little more than a familiarity with the vocabulary. Unfortunately, the formal tone of exposition and the lack of liveliness in the illustrations and format make it unlikely that most non-science students will be spontaneously attracted to the book.

The visual features of Giancoli's book, on the other hand, are unusually attractive. There is an abundance of lively, and often humorous, two-colour illustrations, imaginatively designed to attract attention as well as to give insight into physical principles. The pedagogy and organisation of the text are more conventional, except perhaps for a series of "experiment-projects" to be performed independently by the students. The book attempts to cover a very large number of topics by presenting and discussing results usually without derivation. For a 'physics for poets' course, it would seem preferable to treat a limited number of topics in greater depth.

Hewitt's book has some of the same visual advantages as Giancoli's. In its 650 pages, however, it tends to be even more encyclopaedic, while consciously being even less quantitative. I fear that its admirable (but not subtle) attempt to relate to students with scientifically disadvantaged backgrounds will unfortunately backfire for most liberal arts students. Some of its chapters start with full-page photographs of children with comic-strip-like 'balloons' containing expressions such as "man-o-man-you should've seen the wild spills I used to take before I got into wide wheel bases", before the chapter on rotational motion. The imagination and humour in the book's many small illustrations seem appropriate for all students; the occasional use of a less mature approach and vocabulary may give many students the impression that the author is talking down to them. □

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Introductory meteorology

Ira W. Geer

Fundamentals of Meteorology. By Louis J. Battan. Pp.321. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *The Atmosphere: An Introduction to Meteorology.* By Lutgens and E. Tarbuck. Pp.413. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *A Basic Meteorology Exercise Manual.* Revised edition. By Chelius and Frentz. (Kendall/Hunt: Dubuque, Iowa, 1978.) \$7.95.

THE three publications reviewed here are intended for introductory college-level courses in meteorology for students not majoring in the atmospheric sciences.

By far the best of those examined is *Fundamentals of Meteorology* by Battan. Clearly written and highly readable, it treats topics found in most introductory weather texts and includes chapters on atmospheric optics and acoustics, and weather applications. Reflecting the author's background and interests, frequent referral is made to basic physical principles and relationships which in turn provide the foundation on which subsequent descriptive information is based. Various aspects of air pollution are presented in ways which will no doubt enhance student interest in a number of topics discussed. The book is relatively free from typographical errors or mistatements. However, one is the mention of South Atlantic hurricanes, when hurricanes in the southern North Atlantic

Ocean was obviously intended (page 183). This book is recommended without reservation for use as a course text, a handy teacher reference, or just plain good reading.

The Atmosphere by Lutgens and Tarbuck was an attempt to write a non-technical and non-mathematical treatment of weather and climate. It seems to be geared to the reading and interest levels of the poorly prepared student. Despite the good intentions of the authors, the book fails miserably. It is lacking in scientific accuracy and quality of writing. The concept of (air) pressure is frequently equated to force (pages 10,121,122 and 124). This leads one to conclude that the authors fail to demonstrate an understanding of basic physics, let alone its applications to the atmospheric environment. Statements about "the infinite universe" (page 1) and that "air condenses" (page 233) illustrate the multitude of errors which at best can be called sloppy writing. Simply stated, the book cannot be recommended for any purpose.

A Basic Meteorology Exercise Manual by Chelius and Frentz is "for students of basic meteorology to be used as a supplement to a lecture course or used in an independent laboratory course". Twelve exercises are presented on topics entitled Temperature, Moisture, Winds and Pressure, Clouds and the Precipitation Process, Fronts and Extra-Tropical Cyclones, Upper Air Flow, The Thunderstorm, Climatology, Air Pollution, Weather Radar, Weather Forecasting I and Weather Forecasting II. The bibliography contains a brief list of books and other sources of information.

The manual's sections touching on

satellite imagery, air pollution, weather radar, the relating of surface to upper air weather features, and weather forecasting are somewhat unique as they are not typically treated in the few other basic manuals available commercially. They add a great deal to a publication which is weak in terms of its layout and the quality of its narrative portions. Statements such as "the condensation of water vapour produces precipitation" (page 15) and the description of saturated air as being "like a sponge that can hold no more water" (page 19) appear too often. Unfortunately, student analysis of data from actual atmospheric observations is not often called for. In its present condition the manual cannot be recommended for course adoption. Teachers might find a copy useful for ideas they might want to develop on their own. With considerable revision, the manual could become suitable for general use. □

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Chemistry for the non-science student

A.P. Zipp

Inside Chemistry. By Charles Compton. Pp.569. (McGraw-Hill: New York and London, 1979.) \$16.95.

In the words of the author, this text was written to "meet the special needs and interests of nonscience students" while having enough flexibility to "provide the basis for courses with different emphases, levels and lengths". In my opinion he has been reasonably successful in achieving his goals, although the book has limitations.

On the positive side, the selection of topics seems sufficiently broad to satisfy most instructors, although several lack depth (see below). The text is divided into eight chapters (297 pages) of 'core' material and seven chapters (200 pages) of

'applied' topics, with each of the latter being independent of the others so that they may be studied in any order or combination. In addition, most chapters contain optional sections and the bulk of the quantitative material has been gathered into supplements at the end of the text. These features should allow each instructor to adapt the book to his own course.

The book is written at an appropriate level and the examples used should be of interest to the intended audience. The learning process should be enhanced by the self-study questions (with answers), the practice exercises (without answers), and the key word lists which follow each chapter as well as the excellent glossary at the end of the text. The data and statistics are as current as could be expected, a point which is particularly important for environmental courses, and each chapter has a list of relevant references (some as recent as 1977).

On the negative side, the book is occasionally redundant, although this occurs mostly in the later chapters which are intended to be independent of one

another (for example, photosynthesis is discussed in three different places and production of carbon monoxide in two). More seriously, some discussions lack sufficient depth to be understood readily. Specific examples include Dalton's atomic theory, the greenhouse effect, base pairing in nucleic acids, oral contraceptives, and amino acid balance in foods. Although this can be remedied in lecture, it may be distressing to students who consider a text to be the final authority, and instructors who feel similarly may wish to select a book designed for a course with a particular emphasis (for example, environmental or biochemical).

In summary, this is a potentially useful text with enough strong points to justify its consideration by instructors of non-science major courses but should be recognised as one which will require supplementary work by the teacher in many areas. □

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Aquatic insects

A.A. Grigarick

An Introduction to the Aquatic Insects of North America. Edited by R.W. Merritt and K.W. Cummins. Pp.441. (Kendall/Hunt: Dubuque, Iowa, 1978.) \$21.95.

AQUATIC insects have been receiving a great amount of attention from many scientific disciplines for the past 20 years. During this period a considerable number of taxonomic changes, new information on distribution, and ecological studies on aquatic insects have occurred. Merritt and Cummins have gathered the contributions of an impressive group (21) of scientists to incorporate much of this information into *An Introduction to the Aquatic Insects of North America*. The book was directed at both students and professionals of the many fields that deal with the aquatic habitat and the authors do not assume the reader to have an entomological background.

The first chapter explains the make-up of the book, indicates its limitations and presents an annotated list of general taxonomic references dealing with aquatic insects. Enough general morphology is given in Chapter 2 to lay the groundwork for use of the keys. Chapter 3 is devoted to collecting, sampling and rearing methods which are tabulated by habitat and community. This chapter includes an amazing amount of information in a well organised and concise form and it is well documented with source material and illustrations.

A short chapter on ecology and distribution sets the format for the handling of this information in later chapters at the generic level in each order. The tabular presentation of information includes habitat classification, locomotive behaviour, trophic relations (based on feeding mechanism) and distribution. By necessity, this information is often based on generalisations of limited numbers of species but when it is used in this light and with the supporting references it is of considerable value. In table 4C some of the examples of orders used to demonstrate trophic classification and columns of the classification are either out of alignment or wrongly set up at the printers. The reader should obtain this information directly from the trophic relationships presented at the end of the chapter for each order.

Phylogenetic relationships and evolutionary adaptations of aquatic insects are briefly reviewed in Chapter 5. Chapter 6 takes up the entomological basics of classification and metamorphosis and a key to immatures and adults is given for the orders with aquatic or semi-aquatic insects. These orders are taken up in a phylogenetic sequence in the remaining chapters. A general introduction, pertinent external morphology and keys to the family for immatures and adults are given for each order. Key characters are usually well illustrated and additional taxonomic references follow the keys. Each chapter concludes with a tabular summary of ecological and general distributional information at the generic level.

The keys to Megaloptera, Neuroptera and Lepidoptera (in part) allow identification to genus. About one-third of

the book deals with the aquatic Diptera and includes separate chapters on the Tipulidae, Culicidae, Simuliidae and Chironomidae. The expanded treatment of these families includes keys to immatures and adults to genus for the first three but is limited primarily to tribe for the chironomids. The limitation of the taxonomic treatment to the family level for most orders was disappointing to many researchers and instructors of courses in aquatic entomology. With due respect to the large size of this group of organisms and incomplete knowledge of some taxa (for example, Diptera), the taxonomic information for identification to the generic level has been developed for most families. Identification to genus would certainly be desirable in this book and would appear to have been possible if a comparable amount of effort had been made in this direction as was devoted to summarising ecological information to genus.

As the title states, the region of this book's coverage is North America. However, the great diversity of aquatic insects in North America (over 10,000 species), the degree of ecological coverage, excellent illustrations and extensive source material (1,712 references) make this book very useful to aquatic biologists throughout the world. It is particularly welcomed by instructors of the subject. □

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